ORIGINAL RESEARCH PAPER

Clinical Ophthalmology

AGE-RELATED DIFFERENTIAL EFFICACY ON DRY EYE OF LACTOBIONIC ACID-BASED EYE DROPS

KEY WORDS: Lactobionic acid; hyaluronic acid; dry eye; age

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ABSTRACT

Aim of this clinical trial was to study the age-related efficacy of eye drops containing lactobionic acid (LA) on dry eye symptoms and signs, based on the consideration that dry eye in the elderly is often associated to a decrease of tear lactoferrin, and that LA could be considered a functional mimetic of lactoferrin.

Forty dry eye patients were enrolled, twenty aged between 18 and 55 years, and twenty aged between 56 and 80 years. Each age cluster was randomly divided in two groups: one was treated with hyaluronic acid (HA) eye drops, and the other with a mix of HA and LA. Symptoms and signs were measured at enrolment and up to 45 days.

Both treatments were effective in improving measured parameters in all treated patients. However, the mix with LA appeared to be more effective than HA alone, and such difference in favor of LA was much more evident in the age cluster over 55.

Introduction

According to the most recent DEWS II report *"Dry eye is a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles"*(Craig et al., 2017). The pathophysiology of dry eye disease (DED) allows to discriminate two major types, based on the predominant cause of eye dryness: aqueous deficient and evaporative dry eye, with the latter being predominant (over 75%) on the former (Lemp, Crews, Bron, Foulks, & Sullivan, 2012; Shimazaki, Goto, Ono, Shimmura, & Tsubota, 1998).

From an etiological perspective, there are several recognized risk factors for the development of DED (Stapleton et al., 2017). Sex and age are the most evident, with females generally affected more often than males, especially after menopause. Diabetes, chronic use of certain drugs or surgical interventions, excessive use of video-terminals can all possibly trigger DED. Symptomatic dry eye has been reported to increase progressively with ageing, independently from sex (de Paiva, 2017; Stapleton et al., 2017). About 5-30% of the elderly show signs of eye dryness, the reported frequency being 8.4% below 60 years of age, 15% between 70 and 79 years, and 20% for individuals over 80 years; women, as expected, show a higher prevalence in all age groups (Moss, Klein, & Klein, 2000; "The epidemiology of dry eye disease," 2007).

Among the many physiological changes that accompany ageing, tear film proteins may vary with ageing and dry eye, and very often a decrease in lactoferrin (Lf) and lysozyme (Lz) is observed (McGill, Liakos, Goulding, & Seal, 1984; Seal et al., 1986). In the tear film, Lf accomplishes antinflammatory, antioxidant and antimicrobic activities. The antinflammatory activity is linked to Lf interaction with natural and induced immunity (Flanagan & Willcox, 2009). The antioxidant and antimicrobial activities depend on the iron-chelating ability of Lf, which prevents the formation of iron-dependent hydroxyl radicals that can be generated during an inflammatory response and by microbial infections (Flanagan & Willcox, 2009; Weinberg, 2001). Moreover, since iron is a critical co-factor for bacterial proliferation, the subtraction of iron by Lf has a limiting effect on bacterial growth and survival (Symeonidis & Marangos, 2012).

Lactobionic acid (LA) is a small molecule (MW 358 Da), a polyhydroxy bionic acid made by a sugar (galactose) bound through an ether link to a poly-hydroxy acid (gluconic acid). It is a molecule endowed with several properties that make it an ideal supplement in artificial tears: it is highly hygroscopic and a powerful anti-oxidant (Tasic-Kostov et al., 2012), it is an iron chelator (Isaacson, Salem, Shepherd, & Van Thiel, 1989) and inhibits matrix metalloprotease activity (Upadhya & Strasberg, 2000), it favors wound healing and inhibits inflammatory cytokines expression and bacterial growth (Olivieri et al., in press). These characteristics of LA as described above, make it an ideal substitute for the missing portion of Lf in tear fluid.

Therefore, with the aim of testing a treatment that could be better targeted to age-related dry eye, we set out a pilot clinical trial to verify the ability of a novel eye drop formulation containing an association of hyaluronic acid (HA) and LA to blunt signs and symptoms of dry eye in two population clusters of patients sorted by age (18-55 and 56-80 years old).

Methods

This is a prospective, observational, pilot clinical study, single blind (the operator evaluating patients was not aware of the treatment given), and adhering to the tenets of the declaration of Helsinki. All enrolled patients signed an informed consent before starting treatments.

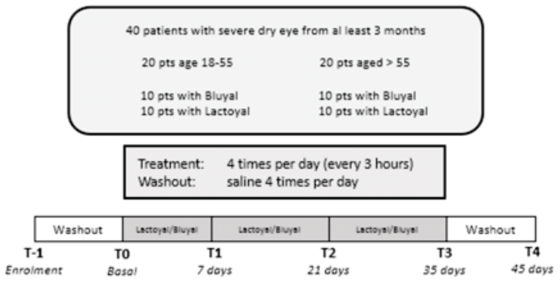
A total of 40 patients affected by dry eye since at least three months were enrolled in the study. Twenty patients were in the age range 18-55 and the other twenty in the age range 56-80. Patients in each age cluster were randomly divided in two groups: one group (10 patients) treated with HA eye drops (Bluyal ® Sooft Italia SpA, Montegiorgio, Italy), and the other group (10 patients) treated with the association of HA and LA (Lactoyal ® Sooft Italia SpA, Montegiorgio, Italy), one drop per eye 4 times a day. Before starting the treatment, patients went through a one-week washout period with saline (Blusal ® Sooft Italia SpA, Montegiorgio, Italy). Treatment lasted 5 weeks, and was followed by a 10-day follow up period. Subjective (VAS and SANDE) and objective (osmolarity, BUT, Schirmer and MMP9) parameters were evaluated soon before starting the treatment (T0) and after 7 (T1), 21 (T2), 35 (T3) and 45 (T4) days (Figure 1). More specifically, osmolarity was measured with the TearLab osmolarity system (San Diego, CA, USA); BUT was detected after fluorescein staining by slit lamp observation; Schirmer type I test (without anesthetic) was performed by using the Contacare Schirmer strips (Gujarat, India) left for 5 minutes in the inferior fornix of the eye; MMP9 was detected by the InflammaDry system (Quidel, San Diego, CA, USA), an antibody based assay which returns a semiquantitative response that can be scored (depending on the intensity of the specific staining) from 0 (no staining: MMP9 < 40 ng/ml) to 1 (barely visible staining), 2 (moderate staining) and 3 (intense staining).

Statistics: A linear mixed model with robust standard error on all sample has been used to evaluate the evolution with time of the different parameters, taking into account repeated measures on the same subject (eyes and time). The interaction of time to group was in the model as fixed effect, subjects and eyes as random effects. The same model was repeated separately for treatment and age cluster. The mean difference with respect to the enrolment value and the respective confidence intervals were obtained after calculation of the marginal means of each group model. No correction was made for multiple comparison. A p value < 0.05 was considered statistically significant. All the analyses were made with the STATA 13.0 software package.

FIGURE 1

Scheme of the clinical trial protocol

SINGLE BLIND ASSESSMENT OF LACTOYAL vs BLUYAL IN THE TREATMENT OF DRY EYE



Results

The characteristics of the 40 patients enrolled in this pilot observational study are described in Table 1. The distribution of sexes was similar in the two age clusters (with a prevalence of women over men) so that the results are not influenced by a different proportion of women vs men. Symptoms measured by the visual analogic scale (VAS), or by the Symptom Assessment in Dry Eye (SANDE) scale (taking into account either the frequency or the severity of discomfort) and ranging from 0 (no discomfort) to 100 (maximum discomfort), were significantly worst in the elderly population than in the younger cluster. Concerning the signs, no difference was evident for tear BUT and for Schirmer, consistent with a severe form of dry eye in the two age clusters, while osmolarity resulted more altered in the younger cluster and MMP9 in the older one.

TABLE 1

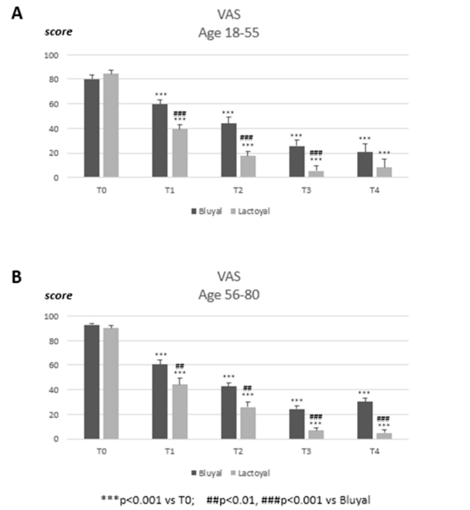
Characteristics of enrolled patients

Age cluster	18-55 (N=20)	56-80 (N=20)
Males	7	6
Females	13	14
	ENROLMENT VALUES (mean ± SE)	
VAS (score)	80.25 ± 2.47	92.8 ± 1.32 ***
SANDE FREQ (score)	79.45 ± 2.72	91.4 ± 1.28***
SANDE SEV (score)	81.9 ± 2.40	92.2 ± 1.24***
SCHIRMER (mm/5')	0.9 ± 0.38	1.05 ± 0.28
BUT (sec)	0.45 ± 0.1	0.5 ± 0.23
OSMOLARITY (mOsm)	348.7 ± 2.92	337.5 ± 2.01**
MMP-9 (score)	1 ± 0.12	1.6 ± 0.16**

**p<0.01 ***p<0.001 vs Age 18-55

The subjective assessment of symptoms measured by the VAS questionnaire (Figure 2) showed a highly significant (p<0.001) progressive improvement with either treatment (Bluyal or Lactoyal) in both age clusters. However, at each time point treatment with Lactoyal gave a significantly better score than treatment with Bluyal in both age clusters. Only at T4 (after a 10-day washout) the score remained significantly better in the elderly, while the difference was lost in the younger cluster.

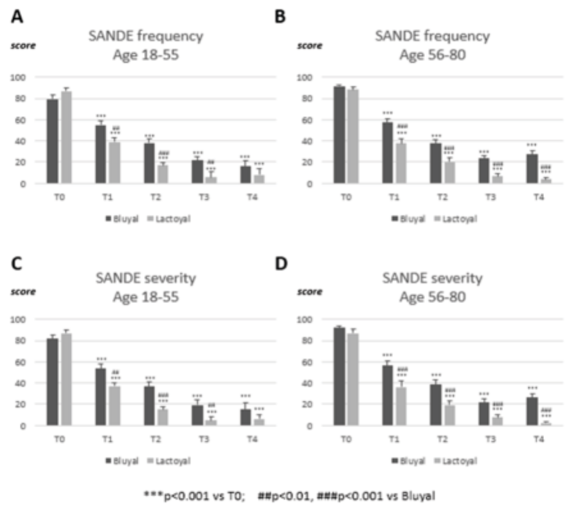
FIGURE 2



Visual Analogic Scale (VAS) evaluation at different time points in the young (A) and the elderly (B) age clusters treated with Bluyal (dark bars) or Lactoyal (light bars)

Similar results were obtained also by the simpler, more direct SANDE questionnaire (Figure 3). The assessment of both frequency and severity of discomfort showed a significant trend of improvement in the two age clusters, with Lactoyal giving better results than Bluyal, with the exception of T4, in which case only elderly responded better to Lactoyal than to Bluyal.

FIGURE 3



Symptom Assessment in Dry Eye (SANDE) evaluation for frequency (A-B) and severity (C-D) at different time points in the young (A-C) and the elderly (B-D) age clusters treated with Bluyal (dark bars) or Lactoyal (light bars). SE bars are shown.

Coming to the objective measurement of signs, Figure 4 shows the progress of Schirmer test with treatment time. In this case a different response of the two age clusters to the treatment was more evident. In fact, although both Bluyal and Lactoyal dramatically improved lacrimation, Lactoyal treatment always gave a better result than Bluyal in the elderly, while a difference in favor of Lactoyal was evident in the younger cluster only at T4.

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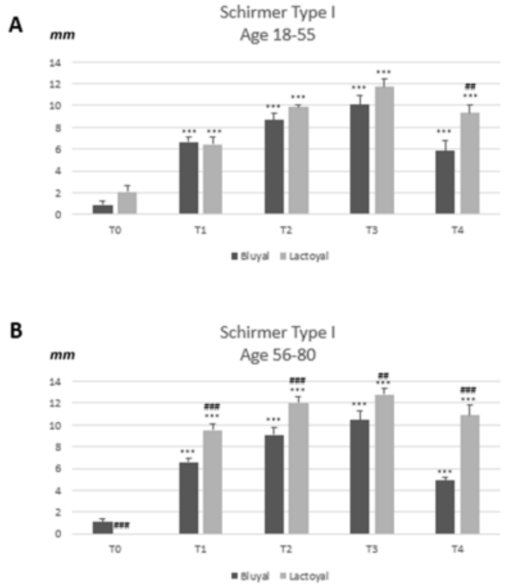
FIGURE 4

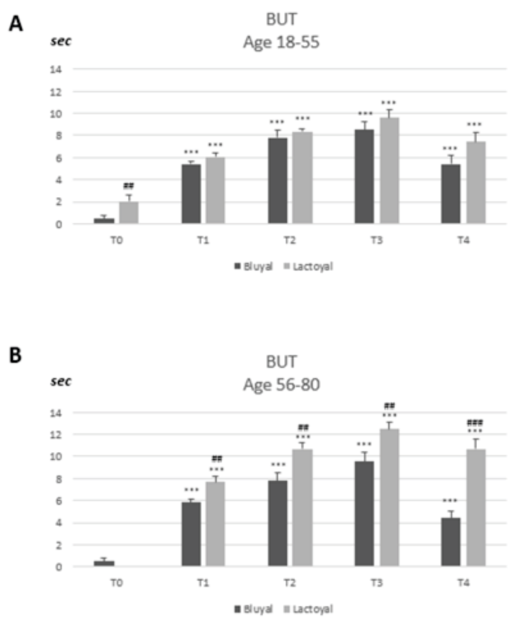


***p<0.001 vs T0; ##p<0.01, ###p<0.001 vs Bluyal

Schirmer type I test evaluation at different time points in the young (A) and the elderly (B) age clusters treated with Bluyal (dark bars) or Lactoyal (light bars). SE bars are shown.

BUT also behaved in a very similar manner (Figure 5). Both Bluyal and Lactoyal drastically improved BUT in the two age clusters. However, while no difference between the treatments was apparent in the younger age cluster, in the elderly treatment with Lactoyal gave better results than treatment with Bluyal at each time point.

FIGURE 5

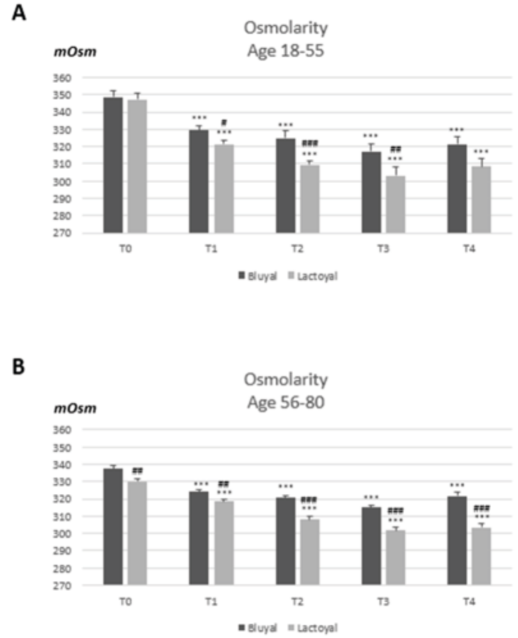


***p<0.001 vs T0; ##p<0.01, ###p<0.001 vs Bluyal

Break Up Time (BUT) evaluation at different time points in the young (A) and the elderly (B) age clusters treated with Bluyal (dark bars) or Lactoyal (light bars). SE bars are shown.

The variation of osmolarity followed a similar tendency (Figure 6), with a significant progressive decrease after either treatment (Bluyal or Lactoyal) in both age clusters, and with Lactoyal being more effective than Bluyal especially in the older age cluster.

FIGURE 6

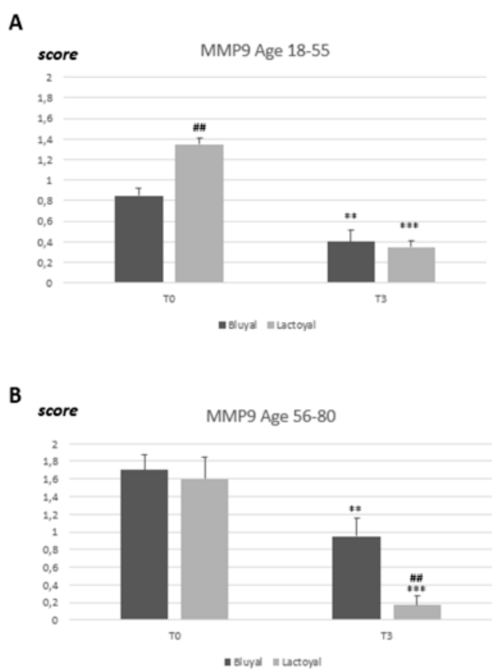


***p<0.001 vs T0; #p<0.05, ##p<0.01, ###p<0.001 vs Bluyal

Osmolarity evaluation at different time points in the young (A) and the elderly (B) age clusters treated with Bluyal (dark bars) or Lactoyal (light bars). SE bars are shown.

Finally, the same trend was confirmed also for MMP9 expression in tears (Figure 7). Bluyal and Lactoyal were equally effective in reducing MMP9 expression in the younger age cluster, while Lactoyal was better effective than Bluyal in the older age cluster.

FIGURE 7



**p<0.01, ***p<0.001 vs T0; #p<0.05, ##p<0.01 vs Bluyal

MMP9 expression evaluation at enrolment and 35 days (T3) in the young (A) and the elderly (B) age clusters treated with Bluyal (dark bars) or Lactoyal (light bars). SE bars are shown

Discussion

Longer life expectancies and age-related conditions leading to increased use of drugs, and changes in lifestyle, all contribute to a progressive increase of ocular surface diseases and eye dryness. However, despite the fact that symptoms and signs of dry eye may appear similar in most instances, its etiology can be different, so that treatments could be more or less efficient depending on whether they are appropriate or not to treat that specific etiology. In fact, nowadays the Italian market offers artificial tears designed for specific needs of dry eye patients, usually based on a hydrating/lubricating agent associated with a specific component better suited to treat certain aspects of the disease, such as echinacea for iatrogenic dry eye (Battiloro, Iannacci, & Lacerenza, 2013); ginkgo biloba for allergic and post-surgical dry eye (Bisantis, 2016; Russo, 2002); liposomes for evaporative dry eye (Gagliano et al., 2014; Lee & Tong, 2012); lipoic acid for diabetic dry eye (Chisari, Stagni, & Di Mauro, 2014; Gomes & Negrato, 2014; Vallenzasca, Fogagnolo, Maggiolo, & Rossetti, 2014). Along this line of thought, a new artificial eye drop containing an association of HA and LA has been proposed, based on the consideration that Lf is deficient in tears of dry eye patients and in the elderly (McGill et al., 1984; Seal et al., 1986), so that dry eye in the elderly might require a higher supply of Lf in tears than dry eye in younger patients. However, artificial eye drops with Lf are not available on the market, most likely due to some instability of the large glycoprotein in the aqueous formulation. LA – a small dimeric polyhydroxy acid – might be a valid alternative to Lf in view of its Lf mimetic activities (see Introduction). We have recently shown in a rabbit model system (Olivieri et al., in press) that LA, with or without HA, favors wound healing in vitro and in vivo. The wound healing assay on rabbit cornea showed that 4% LA in association with 0.15% HA (as in Lactoyal) also resulted in a blunted increase of MMP-9 and TGF-ß in tears and corneal tissue. Finally, the presence of 4% LA resulted in a slower growth of cultured bacterial isolates, as expected with Lf (Comerie-Smith, Nunez, Hosmer, & Farris, 1994; Leitch & Willcox, 1999).

Results obtained in this pilot study and presented in this paper show indeed that the association of HA and LA (Lactoyal®) has a general better efficacy than HA alone (Bluyal®), and its effect is more persistent in time than HA alone. In fact, after 10 days of washout at the end of the study (T4), most of the analyzed parameters remained closer to T3 values with Lactoyal than with Bluyal. Such effects (efficacy and persistency) are even more pronounced in the elderly population with respect to the younger cluster. VAS and SANDE scores at T4 remained significantly better in the elderly treated with Lactoyal (Figures 1 and 2). Lacrimation (Schirmer) and tear-BUT were always more improved by Lactoyal than by Bluyal in the elderly, while the improvement was similar in the younger cluster (Figures 3 and 4). The response in terms of osmolarity and MMP9 expression decrease also appeared more pronounced and persistent in the elderly cluster treated with Lactoyal, while the difference between treatments was less pronounced and even absent at T4 (Figure 6, osmolarity) or at T3 (Figure 7, MMP9) in the younger cluster.

In conclusion, these results strongly suggest that the Lf mimetic activity of LA is critically important to enhance the efficacy of the HA-based eye drop formulation. In fact, the observation that the association of HA and LA appears to be more efficacious than HA alone in the elderly population than in the younger cluster is consistent with the knowledge that in the elderly there is a deficiency of Lf production, that becomes even more pronounced in tears (McGill et al., 1984; Seal et al., 1986). More clinical studies will be necessary on a larger number of patients to confirm these results; however, at this stage of development it is already tempting to propose the association of LA and HA as a tailored-targeted treatment for age-related dry eye, which the ophthalmologist may primarily consider when dealing with this kind of subjects.

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