CASE SERIES

Effects of phytoestrogen supplementation in postmenopausal women with dry eye syndrome: a randomized clinical trial

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ABSTRACT • RÉSUMÉ

Objective: To evaluate the correlation between tear osmolarity and blood levels of 17-β estradiol, estrone, and testosterone in postmenopausal women with dry eye syndrome, and to assess the efficacy and safety of oral supplementation with phytoestrogens, lipoic acid, and eicosapentaenoic acid in this population.

Design: Cross-sectional study including 66 postmenopausal women with dry eye syndrome.

Methods: Sixty-six postmenopausal women with dry eye syndrome were enrolled in a randomized, double-blind, placebo-controlled, crossover study. Patients were divided into 2 groups (groups A and B) and treated, respectively, with phytoestrogen (Bioos, Montegiorgio, Italy) tablets or placebo tablets for 30 days. The 2 treatment periods were separated by a 30-day washout. Patients were examined on days 0 and 30 of each period. Assessments included blood levels of sex hormones, the Schirmer test for tear production, and measurement of tear osmolarity and tear film break-up time.

Results: At baseline, all patients had low sex hormone levels, which were correlated with high tear film osmolarity values ($r = -0.59, -0.61, -0.58$, respectively). After 30 days of therapy, the group treated with Lacrisek® (Bioos) had significantly decreased tear osmolarity ($P < 0.005$) and significantly increased tear production evaluated with the Schirmer test and tear film break-up time values ($P < 0.001$) compared with the placebo-treated group.

Conclusions: Our study confirms that steroid hormones play an important role in ocular surface equilibrium and functions. Consequently, reduced blood levels of these hormones can produce changes at the ocular surface. Phytoestrogen supplementation can significantly improve the signs and symptoms of dry eye syndrome in postmenopausal women.

Objet : Évaluation de la corrélation entre l'osmolarité lacrymale et les niveaux sanguins d'estradiol-17β, d'estrone et de testostérone chez la femme ménopausée ayant un syndrome de sécheresse oculaire, et estimation de l'efficacité et de la sécurité du supplément per os avec phytœstrogènes, acide lipoïque et acide eicosapentaénoïque chez cette population.

Nature : Étude de prévalence incluant 66 femmes postménopausées souffrant de sécheresse oculaire.

Méthodes : Soixante-six femmes en post-ménopause ayant un syndrome de sécheresse oculaire ont été inscrites dans l'étude croisée, contrôlée au placébo, randomisée et à double insu. Les patientes ont été réparties en deux groupes (Groupe A et Groupe B) et traitées respectivement avec des comprimés Lacrisek® ou des comprimés placébo pendant 30 jours. Les deux périodes de traitement ont été séparés par 30 jours de lavage. Les patientes ont été examinées au jour 0 et 30 jours après chaque période. Les évaluations comprenaient les niveaux sanguins des hormones sexuelles, le test de Schirmer pour la production lacrimale et la mesure de l'osmolarité lacrimale et le temps de rupture du film lacrimal (TRFL).

Résultats : Au départ, toutes les patientes avaient de faibles niveaux d'hormones sexuelles, qui étaient corrélés avec les valeurs élevées de l'osmolarité lacrimale (rs −0,59, −0,61, −0,58, respectivement). Après 30 jours de thérapie, l'osmolarité lacrimale du groupe traité au Lacrisek® avait grandement diminué ($P < 0,005$) et la production lacrimale avait augmenté considérablement, selon l'évaluation du test de Schirmer et des valeurs TRFL ($P < 0,001$) comparativement au groupe traité au placébo.

Conclusion : Notre étude confirme que les hormones stéroïdiennes jouent un rôle important dans l'équilibrage et le fonctionnement de la surface oculaire. En conséquence, la réduction des niveaux sanguins de ces hormones peut produire des changements à la surface oculaire. Le supplément phytoestrogène peut améliorer grandement les signes et les symptômes du syndrome de sécheresse oculaire chez les femmes ménopausées.

Dry eye syndrome is caused by a loss of equilibrium between the tear film and the surface of the eye. The deficiency of tear film can be qualitative or quantitative.[1] The sex hormones, in particular sex steroids, contribute to the maintenance of homeostasis at the ocular surface.[1] Androgen and estrogen receptors have been described in the meibomian glands of humans and rats.[2] Androgen and estrogen receptors have also been found in the conjunctival epithelium, on the surface of the cornea, and in the main lacrimal glands.[2] In postmenopausal women, decreases in estrogen production and in the bioavailability of androgen play an important role in the symptoms of the dry eye syndrome.[3] Hormone deficiencies or imbalances alter the fine-tuning regulatory mechanisms that determine the quality of the tear film.[4] Reduced blood levels of sex hormones are associated with tear film instability and increases in tear osmolarity, both of which can lead to dry eye.[4]

The aims of our study were to evaluate the correlation between sex hormone levels and tear film osmolarity in postmenopausal women and to assess the efficacy of an

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oral phytoestrogen supplement in controlling the ocular symptoms of postmenopausal dry eye syndrome.

METHODS

The protocol for this double-blind, randomized, placebo-controlled crossover study was approved by the Ethics Committee of "University Vittorio Emanuele Policlinico Catania." The study was conducted in the Ophthalmic Clinic II, University of Rome "La Sapienza" San't Andrea Hospital, Rome, and in the Ophthalmic Clinic II, Medical University of Catania, Catania, Italy, in accordance with the principles set forth in the Helsinki Declaration and the International Conference on Harmonisation guidelines for Good Clinical Practice. We enrolled 66 postmenopausal women, with interruption of ovarian function of at least 1 year (aged 52–63 years; mean, 57 years) with severe disfunctional tear syndrome (DTS; when symptoms interfere with quality of life). Exclusion criteria were hormone replacement therapy, known hypersensitivity to one of components of the phytoestrogen supplement being tested, autoimmune disease (no patients with Sjögren syndrome were included), and current use of topical anti-inflammatory or antibiotic eye therapy, or both. We enrolled the patients during a project financed by the Italian Ministry of Universities (Gender Bias Project). DTS was diagnosed in accordance with recently published criteria generated by the Dry Eye Workshop.[5] Signs of dry eye considered were bulbar conjunctival vascular dilatation, decreased tear meniscus, decreased tear film break-up time (T-BUT), punctuate epithelial keratopathy, corneal filaments, and corneal ulcera in severe cases.

All the women had stable body weights that were within normal limits (mean weight, 71.6 kg [range, 60.5–80.6]; mean body mass index, 22.5 kg/m^2 [range, 20.0–25.0]). Computerized randomization was used to obtain 2 groups. For the first 30 days of the study, the 33 women in group A were treated with phytoestrogen tablets (Bioos, Montegiorgio, Italy; 1 tablet bid), and those of group B received placebo tablets. After a 30-day washout, the groups were reversed, and group A patients received placebo tablets and group B received Lacrisek (Bioos). Each Lacrisek (Bioos) tablet contained α-lipoic acid (100 mg), eicosapentaenoic acid (240 mg), and extract of fenugreek (200 mg), which contains diosgenin (1.3%), steroidal saponins (50%), and alkaloids, as demonstrated by high-performance liquid chromatography and gravimetry. Placebo tablets were manufactured with the same excipients and were identical to the study supplement in appearance, size, weight, color, and smell. Patients were allowed to use artificial tear products during the study, but they were discontinued at least 24 hours before each study examination.

Participants were examined on days 0 and 30 of each period. Each examination included:

(1) *Body weight:* Patients were weighed with empty bladders and wearing only underclothing. The same scale (accuracy: ±0.1 g) was used for all measurements.
(2) *Blood hormone levels and metabolic parameters:* Blood samples were drawn at 8:00 AM after an overnight fast. Assays included fasting plasma levels of 17-β estradiol, estrone, and testosterone; blood glucose levels; serum triglycerides, total cholesterol, and high-density lipoprotein [HDL]-cholesterol.
(3) *Tear osmolarity:* A 50-nl sample of tear fluid was analyzed with the TearLab Osmolarity System (Tear Lab Corp, San Diego, Calif.). Values ranging from 289 to 308 mOsm/L were considered normal, and those between 312 and 378 mOsm/L were considered indicative of DTS.
(4) *Schirmer test I:* A thin strip of filter paper (35 × 5 mm) was used to quantify tear production over a period of 5 minutes. The strip was placed at the junction of the middle and lateral thirds of the lower eyelid. The patient was instructed to look forward and blink normally during the test.
(5) *T-BUT:* This measures the time interval between a blink and the appearance of a break in the tear film. A fluorescein strip was placed in the lower eyelid fornix and then removed. The patient was instructed to blink three times and then to look straight ahead without blinking. The tear film was observed under a slit-lamp microscope with a cobalt-blue filter.
(6) *Ocular Surface Disease Index (OSDI) questionnaires*: This 12-item, self-administered questionnaire assessed ocular symptoms (photophobia; gritty sensation; eye pain; blurred vision; poor vision; difficulty in reading,

Table 1—Analysis of metabolic parameters in groups A and B before and after 30 days of treatment with Lacrisek® (Bioos, Montegiorgio, Italy) tablets (400 mg/d)

	Group A		Group B	
Parameters	Pretreatment	Post-treatment	Pretreatment	Post-treatment
Weight, kg	72.2 ± 1.9	72.1 ± 2.0	72.5 ± 2.0	72.4 ± 2.0
Triglycerides, mmol/L	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	0.8 ± 0.1
Total cholesterol, mmol/L	4.7 ± 0.2	4.4 ± 0.2	4.9 ± 0.2	4.5 ± 0.2
High-density lipoprotein cholesterol, mmol/L	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1
Fasting plasma glucose, mmol/L	4.8 ± 0.1	4.8 ± 0.1	5.0 ± 0.1	4.8 ± 0.1

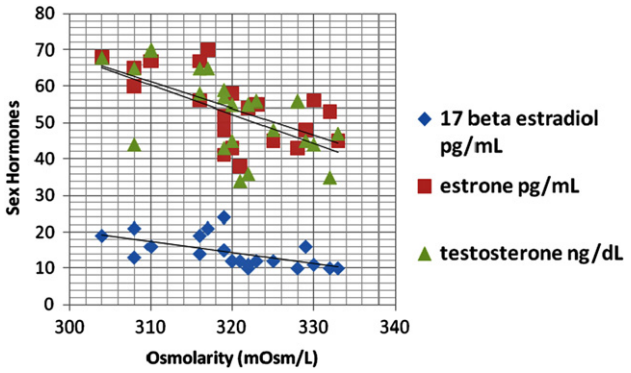

Fig. 1—Relations between tear osmolarity and 17-β estradiol, estrone, and testosterone levels at baseline.

driving at the night, working with a computer or automatic teller machine, or watching television; discomfort under windy conditions, in places with low humidity, or in air-conditioned areas) during the 2- to 4-week period before the examination. Scores range from 1 to 100, with higher scores representing greater disability.

Statistical analysis

Results are expressed as means ± SD. Means were compared using repeated-measures analysis of variance; 95% CIs are given where necessary. Post hoc analyses were performed using the paired *t* test. Calculations and statistical analyses were performed using the SYSTAT version 13.0 software package for Windows (SPSS, Chicago, Ill.).

Results

As listed in Table 1, there were no substantial differences between the 2 groups in terms of pretreatment weight, lipid profiles, or fasting plasma glucose levels, and none of these variables was significantly altered after Lacrisek (Bioos) or placebo treatment.

At baseline (T0), a significant inverse correlation between sex steroid levels (17-β estradiol, estrone, and testosterone) and tear osmolarity was observed in the study population as a whole (day 0; $r = -0.59, -0.61, -0.58$, respectively; Fig. 1).

Table 2 shows tear osmolarity values in the 2 groups (A and B) at baseline (T0), after the first 30 days of treatment (T30), after the 30-day washout period (T60), and at the end of the second (crossover) treatment period (T90). As listed in Table 3, the 2 groups were similar in terms of baseline (T0) T-BUTs, but significant intergroup differences were evident by T30 ($P = 0.000$). Table 4 summarizes the results of the Schirmer test in the 2 groups. The results of OSDI questionnaire showed an improvement of symptoms in patients treated with Lacrisek (Bioos) tablets.

Table 2—Tear osmolarity values recorded during the study period

	Tear osmolarity (mmol/L)		
Time points	Group A	Group B	*P*
T0	361.27 ± 2.16	360.00 ± 4.45	0.14
T30	310.545 ± 6.44	351.181 ± 11.24	0.000
T60	325.181 ± 2.93	360.090 ± 7.21	0.000
T90	330.000 ± 6.88	297.545 ± 9.45	0.046

Table 3—Tear film break-up times recorded during the study period

	Tear film break-up time (sec)		
Time points	Group A	Group B	*P*
T0	0.72 ± 0.761	0.72 ± 0.761	1.00
T30	7.54 ± 2.09	0.90 ± 0.80	0.000
T60	1.72 ± 0.80	1.81 ± 1.35	0.074
T90	0.60 ± 0.78	6.54 ± 2.91	0.000

Discussion

Phytoestrogens are naturally occurring, nonsteroidal plant compounds that are structurally and functionally similar to 17-β estradiol and produce estrogenic effects.[5] They are much less potent than estradiol, but they can exhibit bioactivity when tested at high concentrations.[5] The effects of phytoestrogens also depend on endogenous estradiol levels: When the latter are low, the effects of the phytoestrogens are potentiated.[6] Phytoestrogens interact with estrogen receptors α and β, and display affinity for progesterone receptors and androgen receptors. Estrogens exert both estrogenic and antiestrogenic effects: The former are observed in low-estrogen environments (e.g., menopause, ovariectomy) and the latter in high-estrogen settings.[7] For this reason, phytoestrogens are being increasingly used as an alternative or complement to hormone replacement therapy in postmenopausal women. Postmenopausal hormone changes lead to reductions in the bioavailability of estrogen and androgen. Evidence from several human studies demonstrate that dietary phytoestrogen supplementation can produce mild estrogenic effects in postmenopausal women, including attenuation of hot flushes, reduced osteoporosis, and protection against breast cancer.[6]

It is well-known that hormonal changes in postmenopausal women play an important role in the increased

Table 4—Schirmer test results recorded during the study period

	Schirmer test (mm of strip wetting)		
Time points	Group A	Group B	*P*
T0	1.45 ± 1.32	1.36 ± 1.31	0.780
T30	5.63 ± 3.16	1.45 ± 1.09	0.000
T60	2.00 ± 1.22	1.09 ± 1.18	0.003
T90	1.18 ± 1.04	6.36 ± 2.84	0.000

incidence and prevalence of dry eye in this population. Dry eye is a multifactorial disorder characterized by increased tear osmolarity and inflammation of the ocular surface that causes discomfort and visual alterations.[4] The negative impact of dry eye on quality of life is caused by the ocular irritation, pain, and impaired vision and its effect on general health and well-being.[4]

Our study confirms that steroid hormones play an important role in the ocular surface equilibrium and function. After each 30-day treatment period, tear osmolarity was significantly decreased in the Lacrisek-treated (Bioos) group, but there were no changes in the placebo group. After each period of treatment, T-BUT and tear production (Schirmer test) in patients who had received Lacrisek (Bioos) were higher than those of the group treated with placebo. Differences between pretreatment and post-treatment test results, including OSDI scores, were all statistically significant in the treated group.

In conclusion, our analysis shows that daily intake of lipoic acid and phytoestrogens can be a valid support in the treatment of severe dysfunctional tear syndrome. The use of phytoestrogens also appears to be safe. In fact, no changes in body weight, glucose levels, or lipid profiles were observed after treatment, and no adverse effects were registered. It is important to note that the positive effects of the treatment are transient: In fact, during washout periods, tear osmolarity values increased, and tear production (Schirmer test) and T-BUT values decreased in the group treated with Lacrisek (Bioos).

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