Detection of Oestrogen receptors (ER) α and β in conjunctiva, lacrimal gland, and tarsal plates

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LABORATORY STUDY

Abstract

Purpose Lack of Oestrogen and androgen may be of importance in the pathogenesis of keratoconjunctivitis sicca (KCS). The signal of Oestrogens is transmitted via specific Oestrogen receptors (ER). It was the purpose of this study to evaluate the expression of ER α and ER β in tear-producing tissues.

Methods Reverse transcriptase-polymerase chain reaction (RT-PCR, ER α + β) and immunohistochemical evaluation (ER α only) were performed for ER detection and localization in tissue samples of bulbar conjunctiva (20 samples of 20 patients undergoing cataract surgery), tarsal plates (14 samples of 12 patients undergoing eye lid surgery), and lacrimal glands (20 samples of 13 cornea donors).

Results Messenger RNA ER α was identified via RT-PCR in all tissue samples with variable expression, ER β predominantly in lacrimal gland tissue. Immunohistochemical staining for ER α was negative in most cases, probably due to the thermolability of ERs and very small sample sizes.

Conclusions The detection of ER α and ER β mRNA expression supports the concept of a receptor-based effect of Oestrogen in these tissues contributing to KCS. This may encourage therapeutical efforts including topical Oestrogen administration.

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Introduction

Regarding dry eye syndromes such as keratoconjunctivitis sicca (KCS), more women

than men are affected, especially during pregnancy (predominance of gestagens) and the early phase of menopause.^{1–3} Meibomian gland dysfunction and evaporative dry eye often occur during menopause and aging.¹ In climacteric women, presumably the lower Oestrogen level is responsible for the higher incidence of KCS.^{2,3} Additionally, an attenuation in androgen synthesis during menopause contributes to KCS by leading to meibomian gland dysfunction.¹ Since Oestrogen-dependent cycle-associated histological changes of the conjunctiva have been observed, systemic hormone replacement therapy (HRT) was suggested to be indicated in patients with KCS.² This was supported by investigations in climacteric women with a lower incidence of dry eye complaints under systemic HRT.³ In contradiction, data from the Woman Health Study, a large cohort study with more than 25 000 postmenopausal women, suggested an increased risk of KCS in women who used systemic HRT, particularly Oestrogen alone.⁴ Therefore, the authors of the study recommended not to prescribe HRT for eye symptoms in postmenopausal women.⁴ However, the value of this statement is limited due to the fact that the indications for systemic HRT were not analysed exactly regarding onset and duration of dry eye symptoms. The one and only randomized clinical trial published so far compared patients with and without Oestrogen-containing artificial tears in KCS combined with systemic HRT.⁵ In that study, a statistically significant improvement by topical HRT was demonstrated concerning subjective complaints and objective parameters.⁵ Oestrogen seemed to influence the production of the aqueous component of the tear film.⁵

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The effect of Oestrogen therapy on lacrimal gland, conjunctiva, and/or meibomian glands may be transmitted by Oestrogen receptors (ERs) or in a receptor-independent way. ERs are ligand-dependent transcriptional activators regulating gene expression via complex mechanisms.⁶ Two ER isoforms α and β have been isolated, mapping on different chromosomes (ER α on chromosome 6 and ER β on chromosome 14).⁶ Based on mRNA analyses, ER α is predominantly expressed in specific female tissues, whereas ER β is expressed in many tissues, for example, the central nervous system.⁶ The affinity for Oestradiol is comparable for both receptor subtypes, but ER β seems to be predominant, when both ER β and ER α are present.⁶ Based on different structure and distribution, these ER isoforms are suggested to have different biological activity.⁶ However, function of ER β is not yet understood.⁶

Recent research revealed ER α mRNA as well as androgen and progesterone receptor mRNA in a series of ocular tissue samples ($n=20$) derived from the lacrimal gland, the eye lid and the conjunctiva by RT-PCR.⁷ Regarding ocular tissues, ER β mRNA was detected only in the human cornea and retina to date.^{8,9} With immunohistochemical techniques, ER α could be detected in meibomian glands both in male and female subjects.¹⁰ Depending on age, ER α expression was also proven in the female neurosensory retina and the retinal pigment epithelium.¹¹ Owing to insufficient commercial availability, unsatisfactory characterization and limited applications of ER β antibodies,⁶ only one immunohistochemical study exists regarding distribution of ER β in ocular tissues.⁹ To date, ERs have not been detected in tear-producing tissues using monoclonal antibodies.^{12,13}

Based on pathophysiological and therapeutical considerations, this study was conducted to analyse ER α and ER β expression in the tear-producing tissues.

Materials and methods

After obtaining approval by the local ethics committee and written informed consent biopsies of bulbar conjunctiva (3×3 mm, $n=20$) and tarsal plates ($n=14$) were obtained from volunteers without symptoms of conjunctival, palpebral, or lacrimal gland disease during cataract surgery (20 patients) and lower lid excisions (12 patients), respectively. Lacrimal gland tissue ($n=20$) was taken post mortem from 13 ophthalmologically normal cornea donors on the occasion of cornea removal. All samples were immediately frozen in liquid nitrogen and stored at -80°C until preparation. The study was performed according to the Declaration of Helsinki.

The samples were collected between September 1999 and July 2000. The mean volunteer age was 71.0 years

(38–90 years), the mean cornea donor age was 76.2 (63–89 years). In total, 32 volunteers and donors were female, 13 male (Tables 1 and 2). At the time of tissue sampling, female subjects were elder than males (mean age 75.0 *vs* 68.0 years).

In all, 10 samples of each conjunctival and lacrimal gland tissue and 8 samples of tarsal tissue were analysed by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) for ER α and ER β .¹⁴ After RNA extraction and cDNA synthesis, the expression of the steroid receptors ER α and ER β was determined in comparison to the reference gene GAPDH as an internal standard. The fluorescence labelled PCR products were analysed on an automated fluorescent DNA sequencer (A.L.F., Pharmacia).

Another 10 samples of each conjunctival and lacrimal gland tissue and six samples of tarsal tissue were prepared immunohistochemically for localization of ER α . The Vectastain ABC Elite avidin/biotin/peroxidase kit (Vector Laboratories, Burlingame, CA, USA) in combination with the monoclonal antibody

Table 1 Results of RT-PCR for ER α and ER β sorted by age

No.	Age (years)	Sex	Tissue	ER α mRNA PCR	ER β mRNA PCR	Post mortem time (hours)
1	90	F	Conjunctiva	++	–	none
2	80	M	Conjunctiva	–	–	none
3	79	F	Conjunctiva	++	–	none
4	73	F	Conjunctiva	–	–	none
5	73	F	Conjunctiva	++	–	none
6	72	F	Conjunctiva	+	–	none
7	70	F	Conjunctiva	+	–	none
8	66	F	Conjunctiva	++	+	none
9	46	F	Conjunctiva	–	–	none
10	38	M	Conjunctiva	+	–	none
11	86	F	Lacrimal gland	+	(+)	24
12	84	F	Lacrimal gland	+	++	61
13	83	F	Lacrimal gland	+	(+)	24
14	80	F	Lacrimal gland	+	–	35
15	75	F	Lacrimal gland	+	++	11
16	74	M	Lacrimal gland	+	++	23
17	72	F	Lacrimal gland	+	(+)	23
18	65	M	Lacrimal gland	+	++	21
19	64	M	Lacrimal gland	++	++	39
20	62	M	Lacrimal gland	+	++	12
21	81	F	Tarsal plate	+	–	none
22	81	M	Tarsal plate	+	–	none
23	79	M	Tarsal plate	+	–	none
24	79	F	Tarsal plate	+	+	none
25	76	F	Tarsal plate	+	–	none
26	64	M	Tarsal plate	+	–	none
27	59	F	Tarsal plate	+	–	none
28	58	F	Tarsal plate	+	–	none

–, negative; (+), weak positive; +, positive; ++, strong positive.

NCL-ER-6711/2 (Novocastra Laboratories Ltd, Newcastle, England, UK) was used. Diaminobenzidine (DAB) was applied for staining.

Statistical analysis was performed using SPSS 10. Testing for interdependence of frequency data was conducted using the χ^2 -test.

Table 2 Results of immunohistochemical staining for ER α sorted by age

No.	Age (years)	Sex	Tissue	ER α AB staining	Post mortem time (hours)
29	82	F	Conjunctiva	(+)	none
30	80	F	Conjunctiva	(+)	none
31	80	F	Conjunctiva	—	none
32	79	M	Conjunctiva	—	none
33	78	F	Conjunctiva	—	none
34	77	F	Conjunctiva	—	none
35	75	F	Conjunctiva	(+)	none
36	69	F	Conjunctiva	—	none
37	65	M	Conjunctiva	(+)	none
38	46	F	Conjunctiva	—	none
39	88	F	Lacrimal gland	(+)	19
40	86	F	Lacrimal gland	—	24
41	83	F	Lacrimal gland	—	24
42	82	M	Lacrimal gland	—	23
43	82	M	Lacrimal gland	—	23
44	80	F	Lacrimal gland	—	35
45	75	F	Lacrimal gland	—	11
46	72	F	Lacrimal gland	—	9
47	65	M	Lacrimal gland	—	21
48	64	M	Lacrimal gland	(+)	39
49	93	F	Tarsal plate	—	none
50	93	F	Tarsal plate	—	none
51	64	F	Tarsal plate	—	none
52	62	F	Tarsal plate	—	none
53	59	F	Tarsal plate	—	none
54	45	M	Tarsal plate	—	none

—, negative; (+), weak positive; +, positive; ++, strong positive.

Results

ER α could be found by RT-PCR in 25 of 28 tissue samples with variable expression. ER β was detected in nine samples of lacrimal gland tissue and in one sample each of conjunctival and tarsal tissue (Table 1). Postmortem time did not have any influence on the expression grade. Immunohistochemical evaluation for ER α , however, revealed only a weak staining in four conjunctival and two lacrimal gland samples, but this staining was not focused exclusively to the cellular nuclei (Table 2, Figure 1).

Neither the expression of ER α mRNA or of ER β mRNA nor positive immunohistochemical staining for ER α was associated with age or gender (all differences not statistically significant, Table 3).

Discussion

In this study, messenger RNA ER α was identified via RT-PCR in nearly all samples of conjunctival, tarsal, and lacrimal gland tissue with variable expression. In contrast, ER β mRNA was detected nearly exclusively in lacrimal gland tissue. The mainly negative results of ER β in conjunctiva and tarsal plates may be explained by tissue-specific receptor expression. Since our data showed no statistical significant association of ER α or ER β expression neither with age nor with gender, a presumed loss of expression due to the higher age of most of the volunteers do not seem to play an important role. This is in contrast to the findings of Ogueta *et al*,¹¹ who were the only one, who described an age- and gender-dependent mRNA expression of ER α in a human ocular tissue, here in human neuroretina.

Slight immunohistochemical staining of ER α was demonstrated in four bulbar conjunctival samples and two lacrimal gland samples only (Figure 1). All other

Figure 1 Slice of conjunctiva showing deep brown Diaminobenzidin-labelled positive staining for ER α (arrows). Magnification: 1:200.

Table 3 Distribution of ER α mRNA, ER β mRNA expression and ER α AB staining in association with age and gender

		ER α mRNA		ER β mRNA		ER α AB	
		Negative	Positive	Negative	Positive	Negative	Positive
Gender	Females	2	16	11	7	15	4
	Males	1	9	6	4	6	1
Statistical value, <i>P</i>		0.91 (NS)		0.19 (NS)		0.73 (NS)	
Mean age (years)		66.3	72.4	70.5	73.6	70.0	75.7
Statistical value, <i>P</i>		0.84 (NS)		0.52 (NS)		0.95 (NS)	

All differences were not statistically significant (χ^2 -test). ER: oestrogen receptor; mRNA: messenger ribonucleic acid; AB: antibody; NS: not significant.

samples were negative. In breast cancer, expression of ER is evaluated in a score according to the ratio of stained *vs* unstained cells and the intensity of staining.¹⁵ Since scores for ocular tissues do not exist, such a procedure was not feasible in this study. The negative immunohistochemistry results in this study, which are in contrast to the proof of ER α on the transcriptional level, may have been affected by fixation, embedding, rehydration, and the specificity of the antibody itself.⁶ On the other hand, the absence of immunoreactivity, may also be explained by very low levels of ER α protein below the sensitivity of detection of the assay.¹¹ Furthermore, the method may be unsuitable for ER localization in ocular tissues.

For clinical use, first encouraging results with topical Oestrogen in postmenopausal women have been published.⁵ Oestrogens lead to maturation of the conjunctival surface characterized by preponderance of superficial cells and influence the production of the aqueous components of the tear film.² Hypothetically, presuming a lacrimal gland specific distribution of ER β among the tear producing tissues and a predominance of ER β , when both receptor isoforms are present, this might explain the positive influence of topical oestradiol on the aqueous component of the tear film.^{5,6} Nevertheless, Oestrogens enhance proinflammatory cytokines, such as IL-1, TNF, and/or IL-6 and, therefore, maintain an inflammatory component in KCS.¹⁶ Moreover, it was demonstrated recently that 17 β -Oestradiol induced a marked increase of mRNA levels of IL-1 β , IL-6, IL-8, and GM-CSF in human corneal epithelial cells, contributing to the pathogenesis of ocular surface inflammation in KCS.¹⁷ Androgens, on the other hand, decrease inflammatory processes and regulate meibomian gland function with subsequent improved lipid layer of the tear film.¹⁶ Androgen deficiency, which occurs in postmenopausal women besides Oestrogen deficiency, leads to an altered lipid profile with reduced tear film break-up time and subsequent tear film instability.¹ One case report was published describing successful treatment of KCS in a 54-year-old male resulting in a restored lipid phase of the tear film.^{16,18}

In summary, according to the results of RT-PCR a receptor-mediated effect of Oestrogens via ER α is likely in all three tissues. In lacrimal gland tissue, ER β may play an additive role. Clinically, there may be evidence that KCS especially in elderly women is more probably caused by androgen deficiency rather than by the lack of Oestrogen.¹⁹ Therefore, topical Oestrogen administration might be more effective in combination with topical androgen in KCS.

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