EXTENDED REPORT

Oestrogen-induced changes in biomechanics in the cornea as a possible reason for keratectasia

Eberhard Spoerl, Viktoria Zubaty, Frederik Raiskup-Wolf, Lutz E Pillunat

See end of article for authors' affiliations

Correspondence to:
Eberhard Spoerl,
Department of
Ophthalmology, Carl Gustav
Carus University Hospital,
Fetscherstr, 74, 01307
Dresden, Germany;
eberhard.spoerl@
uniklinikum-dresden.de

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Aim: The risk of regression after photorefractive keratectomy (PRK) and the tendency to develop keratectasia after laser-assisted in situ keratomileusis (LASIK) procedure is higher in women than men. Currently, interest is focused on the influence of oestrogen on corneal stability after corneal refractive surgery. The aim of this experimental study was to investigate the change in biomechanical properties of the cornea induced by oestrogen.

Methods: The influence of oestrogen was investigated in 12 fresh porcine corneas incubated in culture medium with 10 μmol/l β-oestradiol for 7 days. A group of 12 porcine corneas incubated in culture medium without oestradiol for the same time served as a control group. Strips of cornea were cut and the stress–strain was measured in a biomaterial tester. The Young's modulus was calculated.

Results: During incubation the thickness of the cornea changed in the control group by only 6.4% and in the oestradiol group by 12%. However, the difference in the biomechanical stress values at 10% strain was significantly larger. In the control group the stress value was 120.18 ± 28.93 kPa and in the oestradiol group 76.87 ± 34.63 kPa (p = 0.002), representing a reduction of the corneal stiffness by 36% due to the oestradiol treatment.

Conclusion: Oestrogen is a modulating factor of the biomechanical properties of the cornea that is not explainable only by an increased swelling. The significance of the hormone status of patients and its influence on the biomechanical stability of the cornea, a determining factor after refractive surgery, have been underestimated and may contribute to the development of keratectasia.

Changes of the thickness of the cornea due to fluctuations of oestrogen level in blood are known from measurements during the menstrual cycle[1][2] and also during pregnancy.[3] Corneal curvature also changes during pregnancy.[4] Women taking contraceptives[5] report problems with hard contact lenses and pregnant women frequently complain of contact lens intolerance.[6] Currently, much interest is focused on research into the influence of oestrogen on the cornea in relation to corneal stability after corneal refractive surgery, as seen in studies concerning regression after photorefractive keratectomy (PRK) and after laser-assisted in situ keratomileusis (LASIK).[7] The risk of regression after PRK is 13.5 times higher in women taking oral contraceptives.[8] Women taking hormone replacement therapy have a higher risk for post-LASIK refractive regression.[9] Women have a ninefold higher risk for developing keratectasia after LASIK than men.[10]

Since a regression after PRK or the development of keratectasia after excimer laser ablation is related to a reduction of the stiffness of the cornea, it has been postulated that oestrogen possesses a structure-modulating effect on the cornea leading to an alteration of corneal biomechanics. To date, however, no studies have been published regarding the influence of oestrogen on the biomechanical behaviour of the cornea.

Therefore, the aim of this experimental study was to investigate the change of the biomechanical properties of the cornea induced by oestrogen.

METHODS

The influence of oestrogen was investigated in 12 fresh porcine corneas. The corneo-scleral buttons were prepared from porcine eyes enucleated from 4–5-month-old pigs of both sexes of the local abattoir within 5 h post mortem. β-Oestradiol (E2257) (Sigma-Aldrich, Munich, Germany) was dissolved in 100% ethanol to a final stock concentration of 20 μg/ml, which was further diluted to achieve the desired concentration of steroid. The diluted oestrogen solution was added to the corneas in a final working concentration 10 μmol/l. The oestrogen concentration of 10 μmol/l was chosen from other in vivo studies that investigated the influence of oestrogen on lens epithelial cells.[11][12] During pregnancy the oestrogen concentration reaches 100 nmol/l and the pharmacological concentration may reach values up to 10 μmol/l.[12]

The corneo-scleral buttons were excised and organ cultivated in medium MEM (Biochrom AD, Berlin, Germany), containing penicillin (100 U/ml)/streptomycin (100 μg/ml), supplemented with 2% fetal calf serum (Invitrogen, Karlsruhe, Germany) for 7 days at 37°C in an incubator under 5% CO_2 atmosphere. Cell culture media for all samples contained phenol red as pH indicator that allowed visual observation of pH change in the media due to cell metabolism or environment factors. The culture medium in each group was enriched with 4% Dextran T500 (Steromed Biochrom, Berlin, Germany) to avoid swelling of the corneas. The incubation time of 7 days was chosen according to the study of the influence of oestrogen on the skin in mice.[13]

As a control group 12 porcine corneas were incubated in culture medium without β-oestradiol for 7 days. Strips of 10×5 mm were cut from corneas from superior to inferior and the stress–strain relationship was measured with a microcomputer-controlled biomaterial tester (Minimat; Rheometric Scientific GmbH, Benshaim, Germany) at room temperature (22°C). The corneal strips were clamped and a prestress of $5×10^3$ N/m² was applied for 3 min. After that, the strain was increased linearly with a velocity of 2 mm/min and the stress
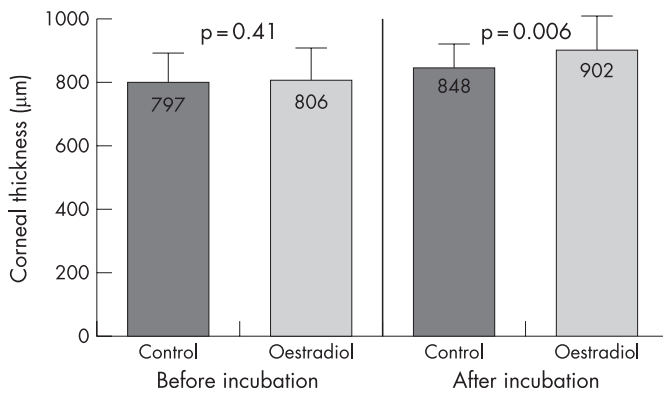
Figure 1 Corneal thickness before incubation and after incubation for the control and oestrogen groups.

was detected. The stress–strain curves were evaluated and the Young's modulus E determined. Measurement of the central corneal thickness using an ultrasonic pachymeter (Pach-Pen XL; Mentor, Norwell, MA, USA) was necessary for the calculation of the stress values. The thickness of the corneas was measured before incubation (original thickness) and after 7 days incubation (thickness after incubation). For statistical calculations the Student's t test was used.

RESULTS

The corneal thickness in the oestrogen group increased during the 7 days of incubation in culture medium containing 10 µmol/l β-oestradiol by 96 µm (12%), from 806 ± 72 µm to 902 ± 98 µm, while the increase of thickness in the control group incubated in culture medium without β-oestradiol was only 51 µm (6.4%), from 797 ± 56 µm to 848 ± 54 µm (Fig. 1). This different increase in thickness between the two groups is statistically significant (p = 0.006).

Fig. 2 illustrates the different stress–strain behaviour of the control and oestradiol-treated corneas. For statistical evaluation of the biomechanics, the stress values were compared at 10% strain. The mean values were in the control group calculated with original thickness 123.54 ± 30.86 kPa (E = 2.9 ± 0.7 MPa), in the oestrogen group calculated with original thickness 88.33 ± 34.38 kPa (E = 2.0 ± 0.8 MPa), in the control group calculated with corneal thickness after incubation 120.18 ± 28.93 kPa (E = 2.8 ± 0.8 MPa), and in the oestrogen group calculated with corneal thickness after incubation 76.87 ± 34.63 kPa (E = 1.7 ± 0.8 MPa). The values of stress and of Young's modulus of the two groups are shown in Figs 3 and 4.
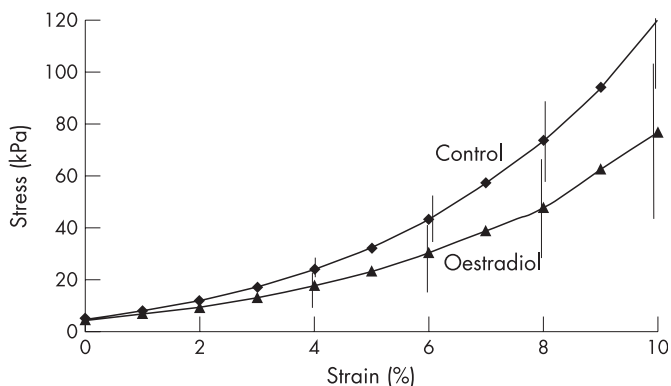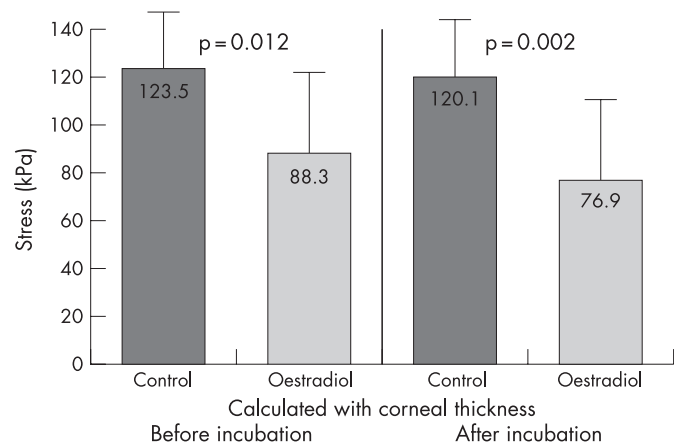

Figure 3 Mean stress values at 10% strain for control and oestrogen groups calculated with corneal thickness before and after 7 days' incubation.

Taking into consideration the change of corneal thickness during incubation, the stress in the oestrogen group was significantly diminished by 36% (p = 0.002) in comparison with the control group. Comparison of the stress of the oestrogen group calculated with corneal thickness before and after incubation shows a reduction of 12.9%, which is not statistically significant (p = 0.371). Thus, the change of thickness alone cannot explain this high biomechanical effect. The main effect of the reduction of the stiffness may be seen from the comparison of the control group and the oestrogen group assuming the original thickness before incubation. In this case the stress is reduced by 28.5% and the Young's modulus is reduced by 30% from E = 2.9 ± 0.7 MPa to E = 2.0 ± 0.8 MPa.

DISCUSSION

This experimental investigation shows a significant influence of oestrogen on the corneal thickness and also on the biomechanics of the cornea. It is the first study showing a direct influence of oestrogen on the biomechanical behaviour of the cornea. Previous reports showed an increase of the corneal thickness[1–3][14] resulting from an elevated hydration.[15] There was a direct linear relationship between hydration and the corneal thickness.[16] All changes in structure of the cornea, such as swelling, influence the mechanical and optical properties and affect the function of the eye.[17] The effect of hydration of the cornea on refraction after radial keratotomy was shown[18] and theoretically explained.[19] The biomechanical stress σ is

Figure 2 Stress–strain behaviour of control and oestradiol-treated corneas.
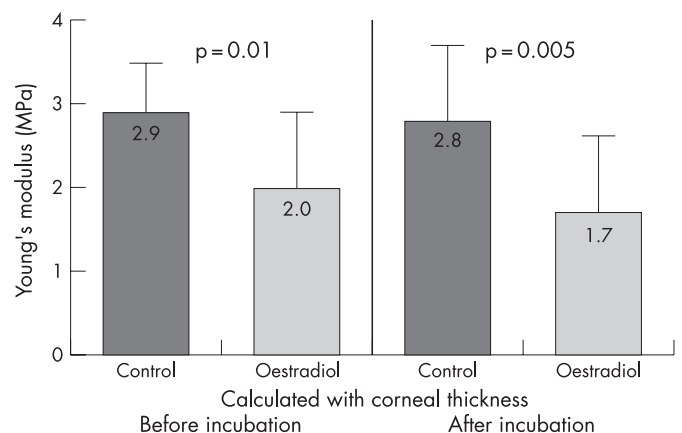

Figure 4 Young's modulus of control and oestogen groups.

calculated by the force F divided by the cross-section area A. Since the cornea swells only in one direction, the swelling is reflected in the corneal thickness. If oestrogen produces only a simple swelling process we would expect a decrease of the stress value of about 6%. However, we found a reduction of the stress of 36%, which means that oestrogen induces further, more complex and deeper biomechanical changes than swelling.

Oestrogen has a stiffness-reducing effect on the cornea that does not play an important role under normal conditions but can be clearly seen in a biomechanically weakened cornea, such as after PRK[8] or LASIK[9][10], and also in cases of keratoconus after puberty[20-24] or after abortion due to the increase of oestrogen activity.[25]

This stiffness-changing effect of oestrogen may be explained at the molecular level. The primary level of action of oestrogen is the regulation of gene expression. Oestrogen has been identified in the tear film and in aqueous humor with a concentration nearly one-half of the plasma concentration.[26][27] It is a steroid hormone acting on specific receptors. Due to its low molecular mass (272.4), oestrogen diffuses into the cells and connects to specific nucleus-receptors. Keratocytes of the human cornea of both sexes contain such oestrogen receptors (OR-α and OR-β), where oestrogen initiates the biochemical changes.[28] The oestrogen–-receptor complex in the cell nucleus then acts as a hormone-induced transcription factor and regulates immediately the gene transcription and thus the intracellular protein synthesis. The increased (or decreased) synthesis of mRNA leads to changed concentrations of proteins in the extracellular matrix as well. Thus oestrogen has a profound influence on composition of cornea. For that reason the cornea is an oestrogen-sensitive tissue, and therefore the biological function of the keratocytes will be influenced by the direct interaction with the hormones. The synthesis of biomolecules is responsible for biomechanical stability and is affected by this cascade.

The stiffness of the cornea is determined by the extracellular matrix consisting mainly of collagens, proteoglycans and glycosaminoglycans.

Oestradiol stimulates the production of matrix metalloproteinases[29], and the production or activation of collagenolytic enzymes may be responsible for the weakening of the collagen network.[30] Oestrogen increases the prostaglandin release and thereby activates collagenases,[31] acting like a chain breaker.[32] This cause a degradation of type I collagen.[33] For the biomechanics of the cornea this means an increased distensibility, but a reduced stiffness.

The viscoelastic behaviour of the cornea is determined also by the proteoglycans and not only by the collagens. Proteoglycans play an important role in the arrangement (distance) of the collagen fibres and in the cohesive force between the lamellae. They act as an ''interfibrillar or interlamellar glue''. It is possible that this cohesion between collagen fibrils and the non-collagenous matrix may be reduced by oestrogen, which facilitates the slippage of collagen within the stroma.[34]

Glycosaminoglycans, as part of the proteoglycans, are responsible for water-binding capacity and for the flexibility of the tissues. Oestrogen stimulates the synthesis of glycosaminoglycans combined with an enriching of water and an increase of corneal thickness. This leads to a weakening of the collagen network.[35-37] The water enrichment seems to be very important in separating the collagen fibres. The oestrogen-induced increasing water-holding capacity of the glycosaminoglycans enables it to act as an osmotic expander, which leads to an increase of the interfibrillar space[17] and mechanical expansion of the crosslinks between the collagen (lamellae or fibrils). Since oestrogen receptors are found also in endothelial cells of the cornea it may be presumed that oestrogen influences the pump function of the endothelium. However, similar changes are observed in the skin, vessel wall or cartilage where endothelium does not play such a function.

From studies on the skin it is known that an increase in the thickness of the skin, combined with an accumulation of water, is the result of the oestrogen-induced synthesis of hyaluronic acid.[38] The water content in the skin can be nearly doubled[13] and the thickness of the skin increases by 7–15% after hormone replacement therapy. In contrast to the cornea, the influence of oestrogen on the biomechanical properties of the skin has already been investigated.[39]

The association between oestrogen and biomechanical modifications has been reported for other biological tissues. Similar reduced biomechanical stiffness was also found at the anterior cruciate ligament in rabbits at higher oestrogen levels.[40]

Not only does an elevated oestrogen level results in biomechanical changes, but also a decreased level. The level of glycosaminoglycans is significantly reduced in cartilage in cases of oestrogen deficiency and this is followed by an increase of cartilage stiffness.[41] From previous studies it is well-known that a degradation of glycosaminoglycans leads to an elevated biomechanical stiffness of tissues.[42][43] In addition, an increase of stiffness with lower compliance of the blood vessels was observed after oestrogen reduction.[44][45]

Previous investigations have shown the relationship between the degree of hydration and the biomechanical properties (especially the viscoelastic behaviour) of the cornea,[35][46] but a connection with an oestrogen-induced changes has not yet been reported. Soergel found a reduction of shearing strength with increasing hydration.[35] Hjortdal[46] revealed in his experimental investigation a significantly increased distensibility of the cornea at higher hydration level.

An increased oestrogen level may be observed during puberty, during the menstrual cycle, during pregnancy, in hormone replacement therapy and in some cases of abortion. It is conceivable that keratoconus may be triggered by oestrogen possibly partly through a genetic predispostion for a weak cornea after puberty.

In a normal pregnancy the oestrogen level increases about by a factor of 20, but the biomechanical effect of oestrogen is widely compensated by the hormone progesterone. Progesterone inhibits prostaglandin production whereas oestrogen increases prostaglandin synthesis. Prostaglandins lead to an increase of collagenases. Progesterone does not only suppress the production of collagenases,[47] but it also inhibits activated collagenases by proteinase inhibitor (tissue inhibitor of metalloproteinases, TIMP).[48] Progesterone insufficiency (especially a low level of progesterone) may lead to abortion, which combined with an elevated activity of oestrogen may also influence the biomechanics of the cornea[25] and cause corneal ectasia. Hormone replacement therapy may also influence the biomechanical stability of the cornea and should be considered in cornea-weakening procedures.

In summary, oestrogen is a modulating factor of the biomechanical properties of the cornea that is not only explainable by an increased swelling. Therefore the influence of the hormone status on the biomechanical stability of the cornea, and even more so on the results of corneal refractive procedures leading to the development of keratectasia, should not be underestimated.

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Authors' affiliations
Eberhard Spoerl, Viktoria Zubaty, Frederik Raiskup-Wolf, Lutz E Pillunat,
Department of Ophthalmology, Carl Gustav Carus University Hospital, Fetscherstr. 74, D-01307 Dresden, Germany

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