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Influence of age, gender and iris color on mechanical and chemical sensitivity of the cornea and conjunctiva

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Abstract

The purpose of this study was to establish the influence of age, gender and iris color on the mechanical and chemical sensitivity of the cornea and the conjunctiva. In 57 healthy subjects (27males, 30 females; ages between 23 and 71 years), sensory thresholds to mechanical and chemical stimulation were measured in the central cornea and the temporal conjunctiva using a Belmonte's gas esthesiometer. Mechanical stimulation consisted of warmed air pulses of 3 s duration at different flow rates (40–200 ml/min). For chemical (acidic) stimulation, 3-s warmed gas pulses containing 10% to 80% CO_2 in air were applied, at a flow below mechanical threshold flow. Corneal and conjunctival thresholds to mechanical and chemical stimuli increased with age. Premenopausal women were more sensitive to corneal stimulation than men of similar ages but overall differences in mechanical and chemical threshold between men and women were not significant. Individuals with blue eyes had significantly lower corneal chemical thresholds than those with brown or green eyes. Multiple linear regression analysis evidenced that corneal mechanical threshold depends on age and iris color according to the equation 58.2 ml/min + 1.3 · (Age − 23 years) −9.7 if blue eyes. For chemical threshold, the equation was 28.6%CO_2 + 05 · (Age − 23 years) − 12.5 if blue eyes. In the case of the conjunctiva, only age determined mechanical and chemical thresholds according to the equations: 78.2 ml/min + 1.4 · (Age − 23 years) and 41.6%CO_2 + 0.8 · (Age − 23 years), respectively. Therefore, normal values of mechanical and chemical thresholds of the cornea and conjunctiva measured with the Belmonte gas esthesiometer can be predicted according to age and iris color. Variations of sensitivity with age, iris color and gender may reflect differences in innervation density and neural responsiveness associated with the hormonal status.

Keywords: corneal innervation; esthesiometry; sexual differences; corneal sensitivity and aging; conjunctival sensitivity and aging

1. Introduction

Measurement of corneal sensitivity with the Cochet–Bonnet and other esthesiometers that stimulate mechanically the ocular surface, have evidenced variations in touch sensitivity associated with age (Jalavisto et al., 1951; Boberg-Ans, 1955; Norn, 1973; Millodot, 1977a; Draeger, 1984; Millodot and Owens, 1984; Lawrenson and Ruskell, 1993; Kohlhaas et al., 1994), hour of the day (Millodot, 1972) or menstrual cycle (Millodot and Lamont, 1974; Riss et al., 1982), although gender differences were not detected (Jalavisto et al., 1951; Millodot, 1975). Intriguingly, it has been also reported that corneal sensitivity to mechanical stimulation varies with the iris color, being higher in blue-eyed individuals (Millodot, 1975).

The psychophysical characteristics of corneal and conjunctival sensibility such as modality, intensity and duration of sensations are primarily determined, at the peripheral nervous system level, by the type and degree of activation by physical and chemical stimuli of the various functional types of sensory receptor fibers innervating these structures. They include mechano-nociceptors, polymodal nociceptors, cold receptors

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and limbal low threshold mechanoreceptors (Belmonte et al., 1997, 2004). Qualitatively distinct sensations are evoked when the different subpopulations of corneal and conjunctival receptors are activated separately or in a variable degree whereas the intensity of the sensation appears to be associated with the number of recruited afferent fibers and the frequency of their discharge (Chen et al., 1995; Acosta et al., 2001a).

The gas esthesiometer developed in our laboratory (Belmonte et al., 1999) allows a more precise and specific activation of the different functional types of sensory afferent nerve fibers innervating the ocular surface by using selective mechanical, chemical and thermal stimulation (Belmonte et al., 1999; Acosta et al., 2001a,b; Feng and Simpson, 2003). The purpose of this work was to apply this instrument to determine the changes in sensitivity of the cornea and conjunctiva that occur with aging, as well as the differences in sensitivity associated with gender and iris color of normal human subjects.

2. Methods

2.1. Subjects

Eyes from 57 normal, healthy subjects of both sexes (27 male and 30 female) of ages ranging between 23 and 71 years, and with different iris color were explored. The iris color was subjectively defined by the experimenter at the beginning of the exploration and classified as brown (35 subjects), green (5 subjects) and blue (17 subjects).

The subjects gave their informed consent and were free to interrupt the session at any time. None of the individuals had ocular pathologies. The research followed the tenets of the Declaration of Helsinki.

2.2. Esthesiometry

Mechanical and chemical thresholds were determined in both eyes, first in the cornea and then in the temporal conjunctiva, using the Belmonte esthesiometer (Belmonte et al., 1999), modified by Holden and co-workers (Stapleton et al., 2004). This instrument delivers a jet of gas to the eye of 3 s duration, containing a mixture of 0–100% CO_2, flowing at controlled rate, between 0–200 ml/min. A modified optical range finder in the probe was used to maintain an alignment distance between the probe and the eye of 4 mm, and two laser pointers assisted with finding the correct working distance when stimulating the conjunctiva (Stapleton et al., 2004). The gas is warmed inside the probe to reach the surface of the cornea at a temperature of 34 °C (Belmonte et al., 1999; Acosta et al., 2001a,b).

The method of levels was used to determine thresholds for mechanical and chemical stimulation (Yarnitsky and Ochoa, 1990; Belmonte et al., 1999; Acosta et al., 2001a,b). For mechanical stimulation, stimuli of increasing flow in 20 ml/min steps were applied until a positive response was reported by the subject. The next stimulus was then applied at a flow rate 10 ml/min lower than the one that had evoked a positive response. If this flow value still evoked a positive response, the following stimulus was reduced by 5 ml/min. Conversely, if it was not felt, a 5 ml/min flow increase was made. The lowest positive response using 5 ml/min steps was taken as threshold. The same procedure was applied for the detection of chemical threshold with CO_2, with a minimum step size of 5% CO_2. Flow rates for CO_2 stimulation were adjusted below the mechanical threshold value (from 40 to 85 ml/min depending on each subject). Two-minute pauses were left between CO_2 pulses in order to avoid chemical sensitization of polymodal nociceptors (Belmonte et al., 1991). At the end of each pulse, subjects were requested to report the presence or absence of sensation, its innocuous or irritant quality and to describe the sensation experienced in their own words.

2.3. Statistical analysis

For each subject, the mean threshold of both eyes was calculated. Pearson correlation was made between age and mechanical and chemical thresholds. *t*-Test was used to find differences between data of male and female subjects and between blue and brown-green eyes. The combined influence of age, gender and iris color on corneal and conjunctival sensitivity was analyzed using Multiple Linear Regression (MLR) analysis. Paired *t*-test and Signed Rank test were used to compare corneal and conjunctival data. The slope and intercept values of corneal and conjunctival mechanical and chemical regression equations were compared using the paired *t*-test and the jack-knife method (first, one data point at a time was removed, using the remaining data to evaluate the model and then examining the fit to the omitted data point; later the slope and intercept means were compared using the paired *t*-test).

3. Results

3.1. Separate influence of age, gender and iris color

3.1.1. Age

Mechanical and chemical thresholds measured at the central cornea and temporal conjunctiva increased progressively with age (Fig. 1). Pearson correlation analysis showed a positive correlation between subject's age, mechanical threshold (Pearson coefficient: 0.808, $P < 0.001$) and chemical threshold (Pearson coefficient: 0.472, $P < 0.001$) in the central cornea. Similar results were obtained with mechanical and chemical threshold values at the temporal conjunctiva (Pearson correlation coefficients: 0.712 and 0.533 for mechanical and chemical threshold, respectively; $P < 0.001$).

3.1.2. Gender

As shown in Fig. 2, threshold values for mechanical and chemical stimuli in the cornea and the conjunctiva appeared to be slightly higher in men in comparison with women, but differences did not reach significance. Nevertheless, when mean threshold values of women were distributed in two groups, premenopausal (<55 years) and postmenopausal (>55 years), and