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Daily rhythm of tear production in normal dog maintained under different Light/Dark cycles

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ABSTRACT

This study was conduced to assess the daily rhythm of tear production in clinically healthy dog. For our study eight female purebred Beagles were subjected to three different Light/Dark schedules: 12/12 L/D, 24/0 L/D and 0/24 L/D cycles. In all subjects Schirmer tear test I was performed at 4 h intervals over a 24 h period. A statistical significant effect of photoperiod was observed comparing the three different L/D schedules, and a statistical significant difference was observed comparing left and right eye during the 12/12 L/D schedule. We demonstrated daily variation of tear production in dogs maintained under an L/D cycle. We also provided strong evidence that the rhythm of tear production is endogenously generated because it persisted in constant darkness. Although the range of excursion of the daily/circadian oscillation in STT I (about 2 mm/min) is likely too narrow to be of clinical significance, it is statistically significant and may have physiological implications not yet appreciated.

1. Introduction

The components of the preocular tear film (PTF) are responsible for maintaining the health and normal function of the ocular surface. Tears help to remove waste products and debris, they provide moisture, lubrication, and essential nutrients, such as oxygen and glucose, to the avascular cornea, and they contain immunoglobulins, enzymes and other proteins which are important for protecting the eye (Gum, 1991; Izci et al., 2002). Reduction in tear production can be due to factors that impact environmental conditions, such as dry air and air conditioning (Wieslander et al., 1999; Doughty et al., 2002); endocrinopathies, such as diabetes mellitus, hypothyroidism and hyperadrenocorticism (Williams et al., 2007); autoimmune diseases (Kaswan et al., 1985); immune-mediate diseases causing inflammatory infiltrate located around the ducts of lacrimal glands, such as leishmaniosis (Narajo et al., 2005); drug toxicity (Tanaka et al., 1983), reaction to antimicrobical drugs and drugs affecting the autonomic nervous system (Collins et al., 1986); topically administered atropine (Hollingsworth et al., 1992) and congenital alacrima (Davidof and Friedman, 1977). Inadequate tear production can result in conjunctivitis, superficial keratitis or corneal ulceration, and impair healing of ulcerated cornea (Brooks et al., 2000). Acute reductions in tear production have been associated with the onset of Keratoconjuntivitis sicca (KCS) (Margadant et al., 2003). In dog, such inflammation may result in corneal ulceration, and, if untreated, even corneal perforation. Loss of vision due to progressive corneal pigmentation is also possible sequela of the disease (Gelatt, 1991).

Traditionally measurement of tear production is based on the Schirmer tear test (STT) I, which measures production of the aqueous portion of the tear film (Gelatt et al., 1975), because of its relatively quick and easy procedure (Hamor et al., 2000). While the STT II is performed after the application of topical local anesthetic and is a measurement of basal tear production alone (Hartley et al., 2006).

Daily fluctuation in STT values has been previously described, but the results have been inconsistent. Berger and King (1998) found a daily and a weekly fluctuation in STT values; Smith et al. (1995) observed a diurnal variation of tear production with highest levels in late afternoon and lowest levels at midday. No statistically significant differences in STT values with time of day were described by Hamor et al. (2000) and Beech et al. (2003). All studies that highlight daily and weekly fluctuation of STT values have been conduced performing STT measurements twice daily (Alkan et al., 2004), at 10:00 AM, 12:00 PM, 2:00 PM and 4:00 PM (Hartley et al., 2006) or in late afternoon and early morning (Berger and King, 1998). These studies showed the existence of STT values variation during the different times of day, but these variations can not be defined as rhythmic. In fact, in these studies an unqualified experimental model has been applied to evaluate circadian rhythm. Circadian rhythm study needs equidistant sampling times (every two, three or four hours) over a 24 h period or more.

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The purpose of this study was to assess the daily rhythm in STT values, to examine tear production in relation to the L/D cycle to see if rhythmicity persists in the absence of an environmental cycle to drive the rhythm, and to establish if there are some differences comparing left and right eye in clinically normal dogs, subjected to three different L/D cycles.

2. Material and methods

2.1. Animals

Tear production was measured using the standard STT I in eight healthy and ophthalmoscopically unremarkable female purebred Beagle, with a mean age of 5 ± 1 years old and mean body weight of 12 ± 1 Kg. We used a single breed (Beagle) and a single sex (female) within a narrow age range to avoid heterogeneity in weight, gender, and age, variables previously shown to affect STT I values (Berger and King, 1998; Hartley et al., 2006).

Prior to the study, complete clinical and ophthalmic examinations were performed in all dogs to determine their health status. Ocular examination included direct ophthalmoscopy, STT I, applanation tonometry, biomicroscopy and flourescein staining. All animals were free of signs of corneal or conjunctival disease and had no history of ocular diseases. They were housed individually at indoor temperature and humidity (18–21 °C; 50–60 Rh%). Ambient temperature and relative humidity for each experimental day were continuously recorded with a data logger (Gemini, Chichester, West Sussex, UK). The shell of the boxes allowed the visual isolation of each dog from cospecifics and avoided the social entrainment of circadian behavioural rhythms (Davidson and Menaker, 2003) All dogs received normal feeding (22.5 g/Kg for each dog of a certified dog diet) provided at 10:00 AM each day. Water was available *ad libitum*. General animal care was carried out by professional staff not associated with the research team. All housing and care conformed to the standards recommended by the Guide for the Care and Use of Laboratory Animals and Directive 86/609 CEE.

2.2. Experimental design

All animals were subjected to three different Light/Dark (L/D) schedules and for 3 days prior to each schedule the animals underwent the same pattern of daily activity: in the first schedule (12/12 L/D period) light timers were set to maintain a L/D cycle with 12 h of light and 12 h of darkness each day (600 lux; lights on at 8:00); during the second schedule (24/0 L/D period) the lights were turned on for all experimental period; during the third schedule (0/24 L/D period) all animals were housed in constant darkness. During each schedule the STT I was performed at 4 h intervals over 24 h period (starting at 8:00 AM on day 1 and finishing at 8:00 AM on day 2). Lighting was uniformly diffused throughout animal box providing sufficient illumination to aid in maintaining good house-keeping practices, adequate inspection of animals, safe working conditions for personnel, and the well-being of the animals. Light was provided by cool daylight fluorescent tubes (FH HE/860 Lumilux T5, Osram GmbH, München, Germany), placed in the middle of the box at 2 m height from the floor. A dim red light (<3 lux, 15 W Safelight lamp filter 1A, Kodak Spa, Milano, Italy) was used to sample dogs during the dark phase. The light intensity was measured by a photometer (PCE-172, PCE Group S.R.L., Lucca, Italy). The same veterinarian and technician performed all the tests.

2.3. STT I assessment

A 35 × 5 mm commercial tear test strip (Schering–Plough Animal Health, Union, New Jersey) was used to record tear production in millimetres wetting per minute, the same lot number of STT strips was used throughout the study to minimize variations relating to the manufacturer. For the routine STT I, the strip was placed just inside the lower eyelid approximately one-third the distance from the temporal to nasal canthus and the eye was gently held closed for one min. Values were recorded from both eyes of each animal. The first eye to be tested was randomly chosen and recorded.

2.4. Statistical analysis

All the results were expressed as mean ± SEM. Data were normally distributed ($p < 0.05$, Kolmogorov–Smirnov test). Two way analysis of variance (ANOVA) was used to compare left and right eyes and to determine the influence of photoperiod on STT I values during protocol testing. One-way repeated measure ANOVA was used to determine a statistical significant effect of time on the tear production for each eye in the different L/D schedules. P values <0.05 were considered statistically significant. The data was analyzed using the software STATISTICA 7 (StatSoft Inc.). In addition, we applied a trigonometric statistical model to the average values of each time series, so as to describe the periodic phenomenon analytically, by characterizing the main rhythmic parameters according to the single cosinor procedure (Nelson et al., 1979). Four rhythmic parameters were determined: mean level, amplitude (the difference between the peak, or trough, and the mean value of a wave), acrophase (the time at which the peak of a rhythm occurs), and robustness (strength of rhythmicity). For each parameter, the mean level of each rhythm was computed as the arithmetic mean of all values in the data set (7 data points); the amplitude of a rhythm was calculated as half the range of oscillation, which in its turn was computed as the difference between peak and trough. Rhythm robustness was computed as a percentage of the maximal score attained by the χ^2 square periodogram statistic for ideal data sets of comparable size and 24 h periodicity (Refinetti, 2004). Robustness greater than 65% is above noise level and indicates statistically significant rhythmicity.

3. Results

Two way ANOVA showed a statistically significant difference comparing left and right eyes ($P = 0.0001$; $F = 24.39$; DF = 1), and a statistically significant effect of photoperiod ($P = 0.01$; $F = 4.52$; DF = 2) on tear production. One-way ANOVA showed a significant effect of time of day on STT I values for eyes during the 12/12 and 0/24 L/D schedules ($P < 0.01$). A statistical significant difference was not found for time of day on STT I values for the 24/0 cycle.

Under the 12/12 L/D condition, left and right eye showed the same trend during the 24 h period of monitoring. But right eye had a statistically higher STT I values than the left eye ($P < 0.002$) (Fig. 1). We had found STT I mean values of 19.29 ± 0.21 mm/min in the right eye and of 20.50 ± 0.32 mm/min in the left eye during the 12/12 L/D cycle, during constant light the right eye showed STT I mean values of 19.61 ± 0.21 mm/min, while the left eye was 20.23 ± 0.09 mm/min, during the constant darkness the right eye showed STT I mean values of 20.12 ± 0.35 mm/min, while the left eye was 20.74 ± 0.32 mm/min. Acrophases were observed at 21:24 for left eye and at 22:19 for right eye during the 12/12 L/D cycle, and at 19:41 for left eye and at 19:42 for right eye during the 0/24 L/D cycle.

Application of the periodic model and the statistical analysis of the cosinor procedure throughout the time series studied in the different experimental conditions, allowed us to ascertain the periodic pattern of the parameter studied in both eyes (Fig. 2).