

Melatonin Concentrations in Aqueous Humor of Glaucoma Patients

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PURPOSE: To determine whether glaucoma patients exhibit an abnormal melatonin concentration in aqueous humor.

DESIGN: Case-controlled study, laboratory investigation.

METHODS: Aqueous humor and plasma samples of 28 patients with primary open-angle glaucoma and 31 non-glaucoma control patients were collected during surgery, and additional plasma samples were taken the night preceding surgery. Melatonin concentrations were determined using direct radioimmunoassay.

RESULTS: This study shows detectable concentrations of melatonin in the aqueous humor of healthy humans (45% of subjects) and of glaucoma patients (36% of subjects) sampled in the morning, with similar levels of aqueous humor melatonin concentrations in both groups (6.4 ± 9.3 standard deviation (SD) pg/ml and 3.6 ± 1.9 pg/ml, respectively). We find no significant association between the severity of glaucoma and melatonin levels in aqueous humor or in plasma.

CONCLUSIONS: Moderate and severe glaucoma does not appear to be associated with abnormal melatonin concentrations in aqueous humor, at least during the morning sampling period assayed in this study. (Am J Ophthalmol 2006;142:325–327. © 2006 by Elsevier Inc. All rights reserved.)

MELATONIN, A LIPOPHILIC INDOLE DERIVED FROM serotonin, is considered to constitute a reliable output marker of pineal cyclic activity, with high nocturnal but low or undetectable diurnal plasma concentrations. In animals, a strong positive correlation exists between the rhythm of intraocular pressure (IOP) and the rhythm of melatonin or the rhythm of N-acetyl transferase, the enzyme necessary for melatonin synthesis.¹ We thus hypothesized that the disturbed rhythmic pattern of IOP in

glaucoma patients would be associated with an abnormal ocular melatonin content.

This study included 59 patients with ($n = 28$) or without ($n = 31$) primary open-angle glaucoma. The control group consisted of age-matched patients without glaucoma but who underwent elective cataract extraction. These experiments were approved by the local Institutional Review Board (CCPPRB Lyon B). Each patient had a complete ocular examination, a questionnaire of general health and ophthalmologic history, and a standardized sleep questionnaire (the Pittsburgh Sleep Quality Index, PSQI). For plasma melatonin, two venous blood samples were taken at midnight the day before the surgery (background light intensity <2 lux, patients were allowed to sleep from 23:00 to 06:30 hours) and at the time of the surgery (before administration of preoperative sedation). For measure of aqueous humor melatonin, samples (100 μ l) were collected at the beginning of surgery. Plasma and ocular melatonin concentrations were determined with a radioimmunological assay (see supplemental material Table).

Control and glaucoma groups were similar according to age, gender ratio, PSQI score, and the type of anesthesia for surgery (Table). This study shows that detectable but low concentrations of melatonin are found in aqueous humor of healthy humans (45% of subjects) and in glaucoma patients (36% of subjects) in the morning (Figure 1). In both the glaucoma and control groups, melatonin concentrations in aqueous humor were not correlated with time of surgery ($P = .4$, Figure 2). The comparison of control subjects and glaucoma patients (Table) for plasma or aqueous humor melatonin concentrations did not reveal a significant difference between both groups. Furthermore, no significant correlation was noted between the severity of glaucoma and melatonin levels in aqueous humor or in plasma ($P = .6$).

In humans, only a single study previously attempted to measure melatonin in aqueous humor,² although the reportedly high levels of melatonin (>2000 pg/ml) were nonphysiologic. Our measures in the aqueous humor of humans are consistent with melatonin levels described in rabbits during the light period.^{1,3}

The absence of a difference between glaucoma and control subjects could be due to the restricted morning sampling period, with confounding factors from antiglaucoma medication, or a lack of relationship between glaucoma and aqueous humor melatonin. The controls show a larger variability of melatonin levels (mainly attributable to three subjects with high concentrations in aqueous humor >15 pg/ml) than expected for the calculation of the number of subjects (SD 6 pg/ml, power 90%, alpha risk of 5%). This implies a reduction of the statistical power of our study. Although the preoperative drugs could potentially have influenced the results, timolol, norepinephrine, and pilocarpine⁴ are not reported to affect the levels of

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Accepted for publication Mar 16, 2006.

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Supported by the Fondation de France (Grant Fouassier), 5th PCRD (QLK6-CT-2002-02258), ACT vieillissement, GIS-Longévité, Région-Rhône-Alpes (Emergence).

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TABLE. Clinical and Biological Data (Melatonin Concentrations) for the Control and the Glaucoma Groups

	Control Group (31 eyes)	Glaucoma Group (28 eyes)	P Value
Age (years)	71.1 ± 9.7	72.6 ± 12.6	.6
% of males	38.7%	28.5%	.5
PSQI score	5.8 ± 3.3	6.06 ± 3.8	.8
Eye (right)	57.6%	46.2%	.4
General anesthesia for surgery	13%	32%	.2
IOP (mm Hg)	15.3 ± 2.2	19.7 ± 6 (under medical treatment)	.001
Melatonin concentrations in aqueous humor (pg/ml), mean ± SD, range	6.4 ± 9.3 (2.6–42.8)	3.6 ± 1.9 (2.6–10.6)	.37
Plasma melatonin concentrations during the surgery (pg/ml)	7.3 ± 6.5 (2.6–26.1)	4.7 ± 4.8 (2.6–23.4)	.14
Plasma melatonin concentrations at midnight (pg/ml)	18.8 ± 14.3 (2.6–55)	23.2 ± 23.9 (2.6–81.5)	.97
Effect of topical beta-blocker on midnight plasma melatonin concentration		- Without: 21.1 ± 30 pg/ml - With: 23.9 ± 22.6 pg/ml	.9
Effect of topical beta-blocker on aqueous humor melatonin concentration		- Without: 2.9 ± 0.6 pg/ml - With: 3.8 ± 2.2 pg/ml	.5
Effect of topical beta-blocker on plasma melatonin concentration during surgery		- Without: 6.0 ± 7.7 pg/ml - With: 4.3 ± 3.4 pg/ml	.5

IOP = intraocular pressure; PSQI = the Pittsburgh Sleep Quality Index (Buysse DJ, Reynolds CFd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res* 1989;28:193–213); SD = standard deviation.

Differences in mean values for continuous variables were analyzed using nonparametric tests (Mann-Whitney and Kruskal-Wallis tests). Proportions were analyzed using a Chi-square test. Values recorded below the assay limit of detection (2.6 pg/ml) were set at the limit of detection. The statistical analysis was performed using the Statistical Package for the Social Science software (SPSS).

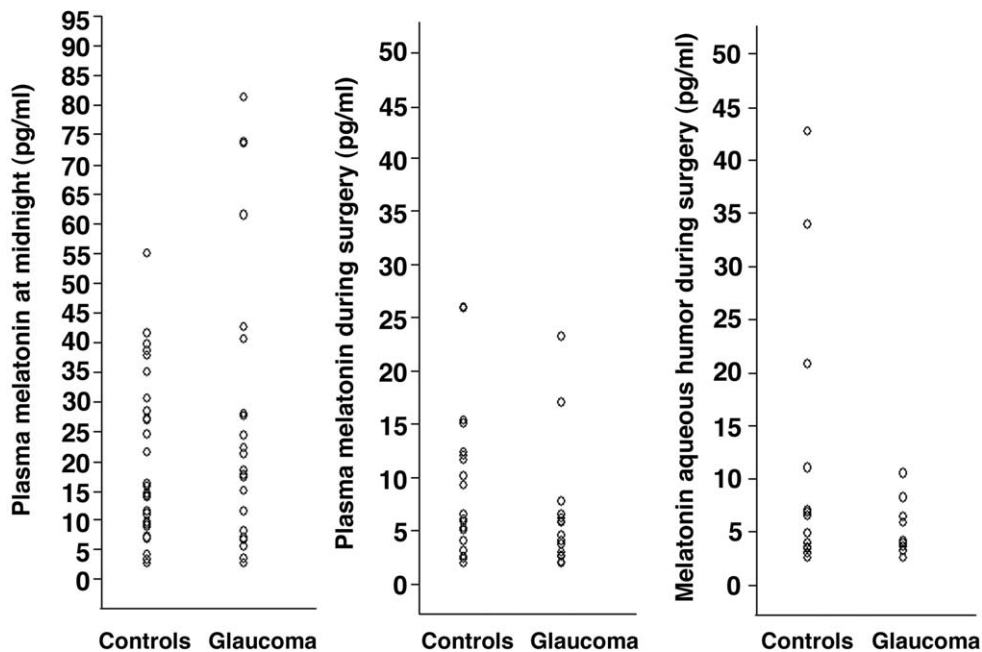


FIGURE 1. Scatter plot of individual melatonin concentrations in control and glaucoma groups, in plasma at midnight (Left), and during surgery (Center) and in aqueous humor at the time of surgery (Right).

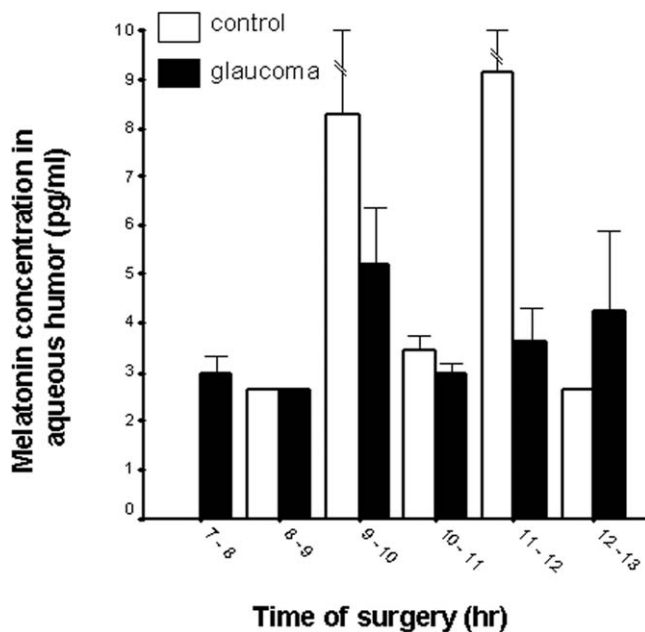


FIGURE 2. Distribution of melatonin concentrations in aqueous humor according to the time of surgery in control and glaucoma groups (error bars = S.E.M; values for controls at 9 to 10 hours = 3.8 and at 11 to 12 hours = 4.1 pg/ml).

melatonin in the rabbit or chicken iris-ciliary body, as suggested by our data for topical β -blocker (Table).

Although a role of melatonin in the regulation of IOP in healthy eyes or during the development of glaucoma cannot be excluded, our results suggest that an abnormal content of melatonin in aqueous humor is not observed in moderate or severe glaucoma. In addition, it cannot be

excluded that the 24-hour rhythm of aqueous melatonin differs in glaucoma patients and in normal subjects. To confirm this possibility, further studies are needed in nonhuman primates over a 24-hour cycle. The reduction of IOP^{5,6} could possibly be mediated via melatonin receptors (as MT₃ receptor) located in the eye, in the anterior segment,⁷ or in the ciliary body.⁵

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SUPPLEMENTARY MATERIAL TABLE. Inclusion and Exclusion Criteria, Characteristics of Glaucoma Patients, RIA Technique

	Control Group	Glaucoma Group
Inclusion criteria	Patients with cataract only.	POAG diagnosed by clinical assessment of the optic nerve head and repeated visual field recordings. Visual fields (Fastpac 24/2, Humphrey™) were graded according to the classification of Hodapp et al. (Hodapp E, Parrish RK, Anderson DR. The asymptomatic patient with elevated pressure. In: Mosby, editor. Clinical decisions in Glaucoma. St Louis: Anderson, D.R., 1993:52–61.)
Characteristics of glaucoma patients		<ul style="list-style-type: none"> - bilateral POAG in 88.5% - mean vertical cup/disk ratio of 0.73 ± 0.2 - mean deviation on the visual field of $-11.4 \text{ dB} \pm 7.4$. - duration of glaucoma: 9.7 ± 6.1 years. - according to the classification of visual field defects by Hodapp et al., 18% of the glaucoma patients had early defects, 60% moderate defects, and 22% severe defects. - topical treatment: β-blocker (75%), prostaglandin (21.4%), α-agonist (14.2%), and/or carbonic anhydrase inhibitor (32.1%). - 60% of patients (17/28) were treated with mono-therapy and 39.2% of the patients (11/28) were treated by bi-therapy before surgery.
Exclusion criteria	previous intraocular surgery or previous argon laser trabeculoplasty, loss of sight in one eye or other ocular disease than glaucoma, psychiatric disorders, hepatic or renal failure, systemic medications (β -blockers, neuroleptic, anti-depressive agents), melatonin absorption, nocturnal work, and recent transmeridian travel (< 3 months).	
Preparation of surgery	- intravenous sedation (propofol) - the pupil was dilated with topical 1% tropicamide and 10% phenylephrine.	
Surgery	Phakoemulsification 100%	trabeculectomy 10.7%, phakosclerectomy 60.7%, phakotrabeulectomy 7.2%, cataract surgery 21.4%
Peribulbar anesthesia	- a 1:1 mixture of 2% lidocaine and 0.75% bupivacaine hydrochloride with hyaluronidase - 78% of the patients	
General anesthesia	- propofol, sufentanil, and sevoflurane - 22% of the patients	
Radioimmunoassay (RIA)	- aqueous humor samples were transferred to a sterile tube in a deep freezer at -70°C until assay. - blood samples were then centrifuged at 4°C , 2000g for 20 minutes, plasma was decanted and stored at -20°C until RIA. - radioimmunological method using a iodinated tracer and antibodies, in duplicate after diethylether extraction of 0.3 ml of plasma or directly from aqueous humor (100 μl). The sensitivity of the melatonin assay was 2.6 pg/ml for aqueous humor and plasma. The intra and inter-assay coefficients of variation were 10 and 14% respectively at a 6 pg/ml concentration for the aqueous humor assay and 5 and 10% respectively at a 25 pg/ml concentration for the plasma assay.	
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POAG = primary open-angle glaucoma; RIA = radioimmunoassay.