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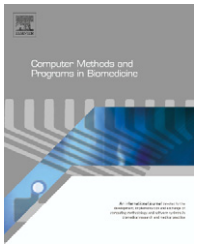

Monte Carlo simulation of latanoprost induced iris darkening

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ABSTRACT

The aim of this study was to provide numerical evidence that latanoprost induced iris darkening (LIID) can be caused by changes to the melanin granule size distribution in the anterior segment of the iris. Iridectomies from two patients were used, where both had undergone unilateral treatment with latanoprost and had exhibited LIID. The untreated eye provided the comparative control. Micrographs from the iris samples were analysed to determine the number and size of the mature melanin granules. Monte Carlo (MC) simulation of light propagation in the iris was performed to examine the changes in reflectance and absorption with varying particle size and density. The reflected intensity of light was obtained as a function of wavelength. CIE colour theory was employed in order to estimate a perceived colour from the reflectance data.

MC simulations showed that the reflectance was reduced for the LIID irises compared to the control irises for both subjects and for all wavelengths of light. The MC simulated colours were in good agreement with the *in vivo* photography of the eye colour. Hence, we have demonstrated that increases in melanin granule size causes iris darkening, and can explain LIID.

1. Introduction

Glaucoma affects between 70 and 90 million people worldwide [1,2] and it is one of the leading causes of blindness in the western world. It is disease of the eye in which the optic nerve is damaged, and vision is impaired. The major cause of this damage is an increase in the intraocular pressure (IOP). Over the last decade, a group of drugs know as prostaglandins have been found to be effective at controlling the IOP by causing an increase in the outflow of aqueous humour from the anterior chamber of the eye. The main prostaglandin drug that is commonly used is the $F_{2\alpha}$ analogue latanoprost (Xalatan®) [3]. An unusual side effect has been observed with all of the topically applied prostaglandin drugs, namely, a darkening of the iris in susceptible individuals. As this side effect was first seen with latanoprost it has come to be known as latanoprost induced iris darkening (LIID). It has been reported that LIID is seen in 22.9% of patients in the UK that received latanoprost [4] and that irises of a mixed colouration (i.e. blue/brown, hazel and golden browns) have a greater tendency to darken.

Although latanoprost induced iris darkening does not affect directly the health of the patient (as far as we are aware), it can affect the quality of life of the patient drastically. This is because of the potentially large and certainly unwanted change in iris appearance that is central to ones self-awareness and identity. We believe that this effect is very important therefore, and that it warrants further study.

Much work has been carried out experimentally in order to understand this darkening effect. Previous experimental studies [5–10] strongly indicate that latanoprost does notstimulate

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cell proliferation. A study (denoted as MainzIIb [9] henceforth) provided the first morphological evidence of the changes that are occurring in LIID cases. This study considered two cases of LIID, and demonstrated that populations of melanocyte cells in LIID cases were stable. However, a detailed analysis of the melanin granules contained in the melanocytes found that there was no increase in the number of melanin granules. The only detectable change that was found was a small, but significant, increase in the sizes of the melanin granules. It has been postulated that this increase in granule size is responsible for the darkening in LIID. However there has been a residual question as to whether such a small change could be totally responsible for bringing about the, sometimes dramatic, observable change of eye colouration that is seen in LIID cases. The purpose of this work is to test this hypothesis theoretically.

As far as we are aware theoretical analysis of the effect of changes to melanocytes, and/or their melanin granules, with respect to iris colour has ever been carried out. Monte Carlo (MC) methods are a standard approach of numerical simulation. The basic methodology of MC methods in simulating scattering of light with human tissues is, by now, strongly established. Thus, much research focussing on the application of MC techniques to specific medical or biologically inspired problems [11–25]. Typical recent examples range from of the spectral response of the skin [17] and ocular fundus [23,24] to that of cancerous tissues [11,15]. In such MC light scattering simulations, this approach employs a 'stochastic' computer simulation technique that involves tracing the flight path of a 'wave packet' of light as it undergoes scattering and absorption in the tissues. The path length and scattering angles are sampled randomly. Overall observable quantities of the tissue, such as the total light reflectance and absorption, are calculated by taking the average over all such wave packets. The objective of this work reported in this article, was to ascertain whether iris darkening can indeed occur because of increased granule size, by using Monte Carlo (MC) simulation.

2. Method

2.1. *Experimental subjects data collection*

The Mainz II study group [6] consisted of 17 patients, all of whom required bilateral surgical intervention (known as a trabeculectomy) to control their elevated IOP. The increased IOP was caused by primary open angle glaucoma (POAG). During the trabeculectomy procedure a small piece of tissue is removed from the trabecular meshwork and the peripheral iris (this is known as an iridectomy) (Fig. 1). At the start of the study the patients had a trabeculectomy on the first eye. The fellow eye was maintained for 6 months on 0.005% latanoprost. Following this period the second eye also underwent a trabeculectomy. Iris photographs were taken at the start of the study and at regular intervals during the study period. Iridectomes from both the surgical procedures, along with the iris photographs, were sent to us in Liverpool for analysis. Ethical approval for the Mainz II study was obtained from the Ethical Review Committee of the Rheinland-Pfalz, and written consent was given by each subject for research use of tissues and

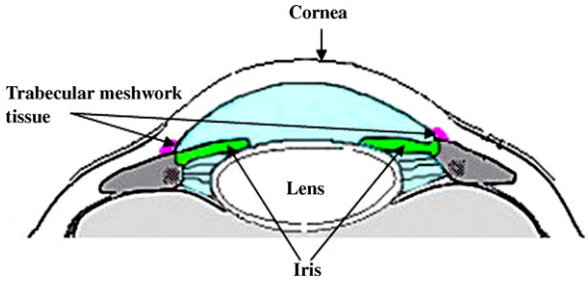

Fig. 1 – Schematic cross section of the anterior chamber of the human eye.

photographs. Of the 17 subjects, only 2 exhibited the LIID side effect (as the individuals were under the care of a Consultant Ophthalmologist we are confident that the darkening of the iris produced was due to the administration of latanoprost, and was not due to any other pathology). These two subjects formed an important sub group known as the MainzIIb study group, which provided a unique opportunity to study the development of LIID in an individual. The small number of subjects is due to the inherent difficulty in collecting data for melanin granule distributions both before and after application of latanoprost to an individual human patient. These two subjects were selected for detailed morphological investigation in order to determine the changes that had occurred in the iris thus resulting in the eye colour darkening [9]. Experimental data from these patients is used in this numerical study, and the patients are referred to, henceforth, as Patient 1 and Patient 2. The irises of both patients were classified by an expert as heterogeneous hazel-coloured for the control irises and homogeneous brown-coloured for the LIID irises.

2.2. *Iridectomy sample analysis*

Iridectomy samples were processed for routine electron microscopy. Initial primary fixation was in 2% gluteraldehyde in 0.1 Sorensons phosphate buffer. This was followed by secondary fixation in 1% buffered osmium tetroxide. The samples were then dehydrated through a graduated series of ethanol, cleared in propylene oxide, and finally embedded in an Aralite/Epon mixture. Thin light microscopy (LM) sections of 1–2 μm and ultra-thin electron microscopy (EM) sections of 90 nm were cut on a Reichart Ultracut E microtone. The EM sections were mounted on copper grids, and counter stained with uranyl acetate and lead citrate.

To ensure the subsequent analysis was unbiased, the tissues were masked at this point. The samples were examined on a Phillips CM10 transmission electron microscope, which was calibrated using a diffraction cross grating replica (Agar S106). A series of 10 micrographs from the anterior border and 10 from the deep stroma were taken for each sample. Contact prints were made from the EM micrographs, which were digitised at a resolution of 150 dpi on a flat bed scanner.

Image analysis was performed using Aequitas IDA software (1.3, DDL Ltd., Cambridge, UK), which was calibrated using the diffraction grating images. The number and size of the melanin granules in the melanocytes were determined for