1. PROTOCOL

AIMS AND HYPOTHESIS(ES) OF THE RESEARCH

Aim: to test the effect of ivabradine, a specific inhibitor of HCN channels, on human retinal cell function assessed by electroretinography.

Hypothesis: inhibition of HCN channels by ivabradine will alter retinal response to light by photoreceptors (assessed by electroretinogram a- and b- wave amplitude and/or latency; and S-cone ERG) or processing by inner retinal layers (assessed by multifocal ERG amplitude and/or latency; and ON- OFF-ERG).

BACKGROUND AND RATIONALE

Hyperpolarization-activated cyclic nucleotide-gated (HCN) ion channels are activated by membrane polarization, and depending on the channel isoform (HCN1-HCN4), modulated by cyclic nucleotides such as cAMP to a varying degree. HCN channels serve important functions in many systems of the body relating to regulation of cell excitability. For example, they act as a cardiac pacemaker in the sinoatrial node (reviewed by Biel et. al. [1] and contribute to oscillatory potentials in the CNS[2]. The current generated by HCN ion channels is known as Ih and If.

All four HCN channel family members are expressed in vertebrate retinal rod and cone photoreceptors, bipolar cells, amacrine and ganglion cells[3], with different cell types having different inventories of ion channels[4 5]. The most established role of photoreceptor HCN receptors in in-vitro studies is to make the light response of rod and cone photoreceptors more transient[6]. In contrast in-vivo targeted deletion of the HCN l channel in the mouse results in prolonged light responses on electroretinographic testing under both scotopic and photopic conditions[7]. These results suggest that HCN channels play a role in modulation of light responses that differs from the key role of the closely related cyclic nucleotide-gated channels in phototransduction[8]. Differences in function of HCN receptors in rod versus cone photoreceptorsand function of HCN receptors in inner retinal cell layers are unclear.

Ivabradine (Procoralan) is a blocker of HCN channels[9]. Ivabradine was approved as the first therapeutic I1i blocker and is used in the treatment of stable angina pectoris[10]. Other known I1i blockers with blocking mechanisms related to that of ivabradine are ZD7288, zatebradine and cilobradine. These were not introduced into therapy because they lacked specificity or exerted unacceptable side effects, in particular visual disturbances due to the inhibition of retinal I1i[11].

[1] Biel M, Wahl-Schott C, Michalakis S, Zong X (2009) Hyperpolarization-activated cation channels: from genes to function. Physiol Rev 89: 847-885.
[2] Siu et al (2006) Hen-encoded pacemaker channels: from physiology and biophysics to bioengineering. J Membr Biol 214: 115-22.
[3] Knop GC et al (2008) Light responses in the mouse retina are prolonged upon targeted deletion of the HCN l gene. Eur J Neurosci 28: 2221-30.
[4] Ivanova E and Muller F (2006) Retinal bipolar cell types differ in their inventory of ion channels. Vic Neurosci 23: 143-54.
[5] Muller F et al (2003) HCN channels are expressed differentially in retinal bipolar cells and concentrated at synaptic terminals. Eur J Neurosci 17: 2084-96.
[6] Barrow AJ and Wu SM (2009) Low-conductance HCN l channels augment the frequency response of rod and cone photoreceptors J Neurosci 29: 5841-53.
[7] Knop GC et al (2008) Light responses in the mouse retina are prolonged upon targeted deletion of the HCN l gene. Eur J Neurosci 28: 2221-30.
[8] Sung C-H and Chuang J-Z (2010) The cell biology of vision. JCB 190: 953-63.
[9] Hofmann F, Biel N, Kaupp UB (2005) International Union of Pharmacology. LI. Nomenclature and structure• function relationships of cyclic nucleotide-regulated channels. Pharmacol Rev 57: 455-62.
[10] Sulfi S and Timmis AD (2006) Ivabradine - the first selective sinus node I(f) channel inhibitor in the treatment of stable angina Int J Clin Pract 60: 222-8.
[11] Bucchi A, Barbuti A, Baruscotti M, Dif rancesco D (2007) Heart rate reduction via selective 'funny' channel blockers. Curr Opin Pharmacol 7: 208-13.

In-vitro studies of blockade of HCN l channels in isolated mouse rods demonstrate selective inhibition by ivabradine, leaving other cellular currents unaffected[12]. Electroretinogram testing of normal and rd 10 mice (a mode of human retinitis pigmentosa) treated with ivabradine showed no effect on electroretinogram a- and b-waves. However reversible changes in response to sinusoidal stimuli were noted during both acute and chronic ivabradine treatment[13].

Patients taking ivabradine may experience visual side effects, thought to be due to blockade of retinal HCN receptors. Phosphene-like effects are reported in up to 15% patients, generally within the first two months of treatment[14 15]. The effects are increased with increasing ivabradine dose. In the largest study of ivabradine so far, 0.3% dropped out because of a composite of eye disorders including phosphenes, blurred vision and visual disturbance[16]. There are no human studies using electroretinography of patients taking ivabradine, particularly those experiencing photopsias. This project aims to perform electroretinographic testing of retinal function of patients who have been prescribed ivabradine by their cardiologist.

This work is important and should proceed because patient symptoms support the concept that ivabradine given for cardiac disease crosses the blood-retina barrier and interacts with retinal HCN receptors. However, at present there are no human studies of the effect of ivabradine on retinal function, although an effect is clearly supported by in-vitro and animal studies. The results will be useful in assessing retinal effects of ivabradine, and will provide information useful for patients taking this medication, particularly if they have established retinal disease prior to taking ivabradine.

Expected benefits to the community are, more fundamentally, a contribution to understanding of retinal cellular function, particularly with regard to HCN receptors.

1.3 METHODOLOGY

Patients will be questioned regarding past ophthalmic history, family ophthalmic history, ivabradine dosage and duration, and other medications taken.

Patients will have a full ophthalmic examination: Visual acuity, refraction, colour vision and eye examination including anterior chamber angle assessment will be conducted prior to pupil dilation (tropicamide 0.5%). If necessary phenylephrine 2.5% will be used to achieve maximal pupil dilation in all participants (>8 mm). A ground electrode will be placed on the forehead and the participants dark-adapted for 20 minutes. After instilling anaesthesia (oxybuprocaine 0.4%) a HK electrode loop will be inserted into the inferior conjunctival fornix of both eyes using dim red light.

Electroretinographic (ERG) testing will involve: Full-field, white flashes of light generated by LEDs (colour temperature 6,500K) will be delivered through a Ganzfield dome using the Roland Consult Electrophysiological Diagnostic System (Roland, Germany). Flashes of 0.01 cdm-2, 3.0 cdm-2 and
10.0 cdm-2 will be used. Participants will be then light-adapted for 5 minutes. Flashes of white light
3.0 cdm-2 individually and flickering at 30Hz will be used. Flash stimuli under dark and light-adapted conditions will be as described in International Society for Clinical Electrophysiology of

[12] Demontis GC et al (2009) Selective Hcn lChannels Inhibition by Ivabradine in Mouse Rod Photoreceptors. IOVS 50: 1948-55. [13] Santina et al (2010) Effect of HCN Channel Inhibition on Retinal Morphology and Function in Normal and Dystrophic Rodents. IOVS 51: 1016-23.
[14] Cervetto L, Demontis GC and Gargini C (2007) Cellular mechanisms underlying the pharmacological induction of phosphenes. Br J Pharmacol 150: 383-390.
[15] Savelieva I and Camm AJ (2006) Novel If current inhibitor ivabradine: Safety considerations Adv Cardiol 43: 95-107. [16] Fox K et al (2008) Ivabradine for patients with stable coronary artery disease and left-ventricular systolic dysfunction (BEAUTIFUL): a randomised, double-blind, placebo-controlled trial. Lancet 372: 817-821.

Vision (ISCEV) protocol for full field ERG[17]. Patients will then be subject to flashes of light 450nm (purple-blue) at 80 cdm-2 on a 612 nm background (red) 560 cdm-2 to differentiate between S and LM-cones[18].Participants will then be subject to 560 nm (green) light at 560 cdm-2 flashes on a background 525 nm (blue) 160 cdm-2 to differentiate between on- and off-bipolar cells[19]. Patients will then be seated in front of a cathode ray tube flickering at 60 Hz, and observe a total stimulus size 30° comprising 61 hexagons retinally scaled, in m-sequence stimulus, mean luminance 60 cdm-2, contrast >90%[20]. All electrophysiological testing is non-invasive. The total test time is 2 hours.

1.3.1 Number, age range and source of participants

Ten adult patients, age range 18 to 55, prescribed ivabradine by their cardiologist will be studied. Twenty matched adult patients, age range 18 to 55, will be studied as controls. Control subjects will have no pre-existing ocular disease and not taking any medications known to affect ERG recordings.The sample size of patients is limited by the small number of patients taking ivabradine in Australia. It is possible that fewer patients will be able to be recruited. The sample size of twenty controls is the minimum recommended by ISCEV, which sets the standard for clinical electrophysiology testing.

As patient and normal data is not normally distributed, statistical analysis will be performed using non-parametric methods. A consultant statistician has agreed to perform statistical analysis.

1.3.2 Informed consent

Subjects will be asked to give informed consent to participate in the study. Informed consent will adhere to the tenets of the declaration of Helsinki.

All subjects will be over 18 years of age and capable of giving informed consent.

1.3.3 Means by which participants are to be recruited

The principal investigator will recruit participants taking ivabradine through their prescribing cardiologist. She has already identified through her clinical practice a suitable patient and the prescribing cardiologist. If insufficient patients are recruited through their cardiologists, advertisements will be placed on her internet site.

Normal subjects will be recruited by advertisement in local newspapers and on noticeboards at the nearby University of Melbourne, subject to approval.

Participants will be paid $20 to assist with out of pocket expenses.

1.3.4 Proposed plan for statistical analysis

As patient and normal data is not normally distributed, statistical analysis will be performed using non-parametric methods. A consultant statistician has agreed to perform statistical analysis.

[17] Doc Ophthalmol 2009; 118: 69
[18] Arden et al Vision Research 1999; 39: 641
[19] Calcagno et al Clin Neurophysiol 2001; 112: 1964
[20] Hood DC et al. ISCEV Guidelines for clinical multifocal electroretinography (2007 edition), downloaded from www.iscev.org

1.4 LAY DESCRIPTION

Participants taking ivabradine for cardiac disease will undergo full field ERG, S-cone ERG, on• off-ERG and multifocal ERG to study their retinal function for predicted abnormalities of photoreceptor, bipolar and inner retinal function caused by ivabradine. They will be compared to normal controls who undergo the same testing protocol. Testing is non-invasive and follows standard ISCEV protocols where applicable.

The visit will consist of a questionnaire completed by the researcher in the participant's presence, and a brief eye examination including measurement of vision. Drops will be placed in the eyes to dilate the pupils, resulting in blurred vision, and the eyes will both be covered by patches for 20 minutes to allow dark adaptation.

After 20 minutes participants are taken into the testing room for ERG testing. Under dim red lighting (like a photographic dark-room) the eye patches are removed and an electrode positioned next to both eyes and on the forehead. Participants then watch white flashes of light which gradually increase in brightness. The room light is then turned on and participants watch more lights, both white and coloured blue and green on coloured backgrounds. Participants then watch a honeycomb pattern of lights on a TV screen. The responses of the retina while watching the flashing lights are recorded.

The preliminary questionnaire and examination takes about 10 minutes, dark adapting 20 minutes and ERG testing about 90 minutes. The total time attending the clinic is 2 hours.

Electroretinography testing is an accepted clinical test, including accepted for clinical use by Medicare Australia, and performed according to accepted international standards (ISCEV). Testing is non-invasive.

DISSEMINATION OF RESULTS

It is intended to publish the research in Documenta Ophthalmologica, the journal of ISCEV.

The data will be owned by the Principal Investigator. The normal data will be of value in future studies.

Patients taking ivabradine who complete the study will have a copy of their electrophysiology results and interpretation provided to their cardiologist.

Participants will be provided with a letter summarising published results and/or a copy of the accepted publication upon request.

1.5 OTHER APPROVALS REQUIRED?

No other approvals are required.