Available online at www.sciencedirect.com
SCIENCE DIRECT®
Experimental Eye Research 82 (2006) 427–440
EXPERIMENTAL EYE RESEARCH
www.elsevier.com/locate/yexer

Morphometric evaluation of changes with time in optic disc structure and thickness of retinal nerve fibre layer in chronic ocular hypertensive monkeys

Masamitsu Shimazawa[a,c], Goji Tomita[b], Takazumi Taniguchi[a], Masaaki Sasaoka[a], Hideaki Hara[a,c,*], Yoshiaki Kitazawa[d], Makoto Araie[b]

[a]*Glaucoma Group, Research and Development Center, Santen Pharmaceutical Co. Ltd, Nara, Japan*
[b]*Department of Ophthalmology, University of Tokyo School of Medicine, Tokyo, Japan*
[c]*Department of Biofunctional Molecules, Gifu Pharmaceutical University, 5-6-1 Mitahora-higashi, 502-8585 Gifu, Japan*
[d]*Department of Ophthalmology, Gifu University School of Medicine, Gifu, Japan*

Received 3 March 2005; accepted in revised form 1 August 2005
Available online 4 January 2006

Abstract

We examined the time course of changes in optic disc structure by means of a scanning laser ophthalmoscope (Heidelberg Retina Tomograph, HRT) in ocular hypertensive (experimental glaucoma) monkeys, and clarified the relationships between the histological RNFL thickness and HRT parameters. Further, the time course of changes in retinal nerve fiber layer (RNFL) thickness in individual eyes was measured using a scanning laser polarimeter with fixed corneal polarization compensator (GDx FCC). In the present study, two separate experiments were carried out. A chronic intraocular pressure (IOP) elevation was induced by laser trabeculoplasty in the left eye in 11 cynomolgus monkeys. In Experiment 1, the HRT and GDx parameters were measured 12 weeks after the laser treatment in 10 eyes in five monkeys. In Experiment 2, the time course of changes in the HRT and GDx parameters was examined before and 1, 3, 4, 5, 6, 8, 10, 12, 14, and 16 weeks after the laser treatment in 12 eyes in six monkeys. The retardation values (thickness parameters) obtained from the GDx were used to derive thickness and ratio parameters in the superior, inferior, nasal and temporal quadrants. Ratio parameters were expressed as a ratio of superior and inferior quadrant to nasal quadrant. After the last measurements, each eye was enucleated, and retinal cross sections were prepared for histological analysis.

In the left (hypertensive) eyes, IOP was persistently elevated throughout the observation periods in both Experiments 1 and 2. In the HRT measurements in Experiment 1, seven out of eight global topographic parameters (exception, disc area) were statistically different between the hypertensive and control eyes 12 weeks after the laser treatment. In Experiment 2, the HRT parameters changed in a time-dependent manner, but each of them almost plateaued at about 4 weeks after the laser treatment. Significant correlations were seen between the histological mean RNFL thickness at 1.5 disc diameters from the optic disc margin and the HRT parameters in 21 eyes from 11 monkeys in Experiments 1 and 2. Especially good correlations with histological mean RNFL thickness were seen for the rim volume and cup volume.

In Experiment 1, good correlations were found between GDx ratio parameters and histological RNFL thickness in individual right control eyes ($n=5$). In individual left experimental glaucoma eyes of Experiment 2 ($n=6$), GDx ratio parameters declined in a time-dependent manner alongside the IOP elevation.

In conclusion, alongside the IOP elevation, time-related changes in optic disc topography and RNFL thickness were demonstrated in monkey eyes using HRT and GDx. HRT (rim and cup) parameters showed good correlations with histological RNFL thickness, and significant interrelations.

Keywords: ocular hypertensive monkey; optic disc; retinal nerve fibre layer; scanning laser ophthalmoscope; scanning laser polarimeter

1. Introduction

Open-angle glaucoma (OAG) is a slowly progressive and chronic disease. To diagnose OAG, especially in its early stage, evaluation of the appearance of the optic nerve head and peripapillary retina is most important. For objective

* Corresponding author. Dr Hideaki Hara, Department of Biofunctional Molecules, Gifu Pharmaceutical University, 5-6-1 Mitahora-higashi, Gifu 502-8585, Japan.
E-mail address: hidehara@gifu-pu.ac.jp (H. Hara).

0014-4835/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.exer.2005.08.001

examination of these structures, the confocal scanning laser ophthalmoscope (Heidelberg retina tomograph, HRT; Heidelberg Engineering GmbH, Heidelberg, Germany) and scanning laser polarimeter (GDx; Laser Diagnostic Technologies, San Diego, CA, USA) have been developed. Analysis of HRT images allows quantitative three-dimensional optic disc topography (e.g. measurement of cup volume and rim volume), and HRT has been widely used as a research tool in topographical analysis of the optic nerve head (Malinovsky, 1996). The GDx is a scanning laser polarimeter for measuring the thickness of the peripapillary retinal nerve fibre layer (RNFL), which is directly affected by glaucoma, and this instrument can provide an objective assessment of optic nerve fibre layer thickness. The data obtained from these devices show correlations with the visual field defect in patients with OAG (Iester et al., 1997; Chen et al., 1998), and they can discriminate with certain sensitivity and specificity between eyes with glaucoma and normal eyes (Weinreb et al., 1995a; Essock et al., 2000; Wollstein et al., 2000).

Experimentally, the laser-induced ocular hypertensive monkey is a widely used animal model of glaucoma (Pederson et al., 1984; Fukuchi et al., 1992; Quigley et al., 1996). Morgan et al. (1998) reported that in a single normal monkey, the value for RNFL thickness obtained from the Mark II Nerve Fiber Analyzer (NFA, Laser Diagnostic Technologies), a prototype GDx, correlated with the histological one, while Yücel et al. (1998) noted that HRT parameters correlated with the number of optic nerve axons in 10 laser-induced ocular hypertensive monkeys. However, these studies were carried out after the glaucomatous changes had been established, and there is little information in the literature as to how the RNFL thickness alters alongside the IOP elevation. In a longitudinal study, Burgoyne et al. (1995) made a detailed examination of optic disc surface changes using digital ocular funduscopy and confocal scanning laser tomography (TopSS, Laser Diagnostic Technologies), and concluded that early changes in the optic disc surface are unlikely to be due to axon loss alone, but to damage to the load-bearing connective tissues of ONH. However, to our knowledge, no study has yet examined both the optic disc surface and RNFL thickness changes with time alongside an IOP elevation. Further, few studies have compared the in vivo HRT findings obtained just before sacrifice with findings derived from post-mortem histological examination of the retinal nerve fibre layer.

The current study had two aims. One was to examine how optic disc topography and RNFL thickness might alter alongside a chronic IOP elevation in laser-induced ocular hypertensive (experimental glaucoma) monkey eyes. The other was to compare optic disc topography (as determined using HRT) just before sacrifice with the histological findings obtained in the same eyes in a large enough number of monkey eyes for the comparison to be scientifically valid.

2. Materials and methods

2.1. Animals

A total of 11 young adult cynomolgus monkeys (*Macaca fascicularis*) weighing 4.0–6.0 kg aged 5–6 years (Keari Co. Ltd, Osaka, Japan) obtained from the same colony at the same time were used to keep the animal conditions uniformly. They were housed in an air-conditioned room at 24 ± 2 °C with $60 \pm 10\%$ humidity, and given food and water ad libitum. All investigations were in accordance with the guideline of the Statement on the Use of Animals in Ophthalmic and Vision Research, and were approved and monitored by the Institutional Animal Care and Use Committee of Santen Pharmaceutical Co. Ltd.

2.2. Induction of experimental ocular hypertension (experimental glaucoma)

An elevation of intraocular pressure (IOP) was induced in each monkey by applying the argon-laser photocoagulation burns to the trabecular meshwork of the left eye, with the right eye being used as an untreated control, as previously described (Quigley and Hohman, 1983). The laser irradiation was performed only on the left eyes, because we considered that laser irradiation on the ipsilateral eyes under the same conditions would facilitate accurate irradiation without technical dispersion. For the laser treatment, the animals were anaesthetized with an intramuscular injection of ketamine (8.75 mg kg^{-1}, Ketalar 50®; Sankyo, Tokyo, Japan) plus xylazine (0.5 mg kg^{-1}, Celactal®; Bayer, Leverkusen, Germany). Then, a single-mirror Goldmann lens filled with a hydroxyethylcellulose solution (Scopisol®15; Senjyu Pharmaceutical, Osaka, Japan) was placed on the eye to be treated. An argon blue/green laser was focused on the mid-portion of the trabecular meshwork, and a total of 150 laser-beam spots were applied around 360° (spot size, 100 μm; power, 1000 mW; exposure time, 0.2 sec) using an argon-laser photo-coagulator (Ultima 2000 SE®; Coherent, Inc., CA, USA) attached to a standard slit-lamp microscope (BQ 900; HAAG-STREIT, Köniz, Switzerland). Two weeks after the first treatment, the laser treatment was repeated so as to produce a maintained elevation in IOP. Time (in weeks) ‘after the laser treatment’ should be understood to date from the first of these treatments.

2.3. Experimental procedure

In this study, two separate experiments were carried out. In the first series (Experiment 1), five experimental glaucoma monkeys were used for comparison of the HRT and GDx results and fundus photographs with postmortem histological findings obtained 12 weeks after the laser treatment. IOP was measured (see below) before and at 3, 4, 6, 8, 10, and 12 weeks after the laser treatment. Baseline