Experimental Eye Research 93 (2011) 82–90

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Correlation of optical coherence tomography with clinical and histopathological findings in experimental autoimmune uveoretinitis

Ivana Gadjanski [a,b,c], Sarah K. Williams [d,*], Katharina Hein [a], Muriel B. Sättler [a], Mathias Bähr [a], Ricarda Diem [d]

[a] Department of Neurology, Georg-August University, Robert-Koch Str. 40, 37075 Göttingen, Germany
[b] Department of Biochemistry and Medicine, State University Novi Pazar, Novi Pazar, Serbia
[c] Research and Development Center for Bioengineering, Sretenjskog ustava 27, Kragujevac, Serbia
[d] Department of Neurology, University of the Saarland, Kirrberger Strasse, 66421 Homburg/Saar, Germany

ARTICLE INFO

Article history:
Received 26 January 2011
Accepted in revised form 29 April 2011
Available online 11 May 2011

Keywords:
experimental autoimmune uveoretinitis
rats
in vivo imaging
optical coherence tomography
histopathology

ABSTRACT

Optical coherence tomography (OCT) is becoming the state-of-the-art method for the non-invasive imaging of a variety of ocular diseases. The aim of this study was to assess the application of OCT for the *in vivo* monitoring and follow-up of pathological changes during experimental autoimmune uveoretinitis (EAU) in rats. Initially we established OCT imaging in healthy brown Norway rats and correlated it with retinal histology. Subsequently, we induced EAU and imaged animals by OCT throughout the pre-peak, peak, and post-peak phases of the disease. The sensitivity of OCT imaging was determined by comparison with clinical EAU and histopathology scores obtained *ex vivo* at several time points throughout the disease course. Our data demonstrate that OCT imaging of the healthy rat retina closely correlates with histological observations and allows the clear visualization of all retinal layers. After induction of EAU, the first pathological changes could be detected by OCT at day (d) 8 post-immunization (p.i.) which corresponded to the time point of clinical disease onset. An increase in retinal thickness (RT) was detected from d10 p.i. onwards which peaked at d16 p.i. and decreased again to near control levels by d20 p.i. We introduce a novel semi-quantitative OCT scoring which correlates with histopathological findings and complements the clinical scores. Therefore, we conclude that OCT is an easily accessible, non-invasive tool for detection and follow-up of histopathological changes during EAU in rats. Indeed, significant differences in RT between different stages of EAU suggest that this OCT parameter is a sensitive marker for distinguishing disease phases *in vivo*.

1. Introduction

Experimental autoimmune uveoretinitis (EAU) is an animal model of autoimmune uveitis, a group of diseases which target the uvea and retina. The main features include retinal and choroidal inflammation, vasculitis, photoreceptor destruction, and ultimately, loss of vision (Caspi et al., 1996; Chan et al., 1990). EAU can be induced in various animal species by active immunization with different retinal antigens, such as retinal soluble antigen or inter-photoreceptor retinoid-binding protein (IRBP) (Adamus and Chan, 2002), or by adoptive transfer of autoreactive T cells (Caspi et al., 1986; Caspi, 2003).

Longitudinal *in vivo* studies of the pathological changes during EAU are necessary for elucidation of the disease-causing mechanisms and for monitoring disease onset and progression during therapeutic intervention. In order to perform such examinations, non-invasive, accurate techniques, which allow for repetitive reproducible imaging, should be used. Recently, a number of studies have indicated that optical coherence tomography (OCT) is becoming the state-of-the-art imaging technique for performing high-resolution, cross-sectional ophthalmic imaging (Fujimoto et al., 2000; Li et al., 2001; Grieve et al., 2004; Srinivasan et al., 2006; Ruggeri et al., 2007; Sakata et al., 2009). OCT is especially useful in serial observations of disease development, because it can provide images of tissue *in vivo* and in real time, without the need for excision and processing of specimens (Fujimoto, 2003).

However, up to now, no studies have assessed the efficacy of OCT for monitoring EAU in rodents. In the present study, we used EAU not only as a model for autoimmune disease, but also as

* Corresponding author. Department of Neurology, University of the Saarland, Kirrberger Strasse, Building 61.4, 66421 Homburg/Saar, Germany. Tel.: +49 6841 1647872; fax: +49 6841 1647801.
E-mail address: sarah.williams@uks.eu (S.K. Williams).

0014-4835/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.exer.2011.04.012

a paradigm for assessing the efficacy of OCT to visualize and monitor progressive changes in the retina and uvea *in vivo*, in brown Norway (BN) rats immunized with IRBP. The *in vivo* OCT images from different time points were compared with those obtained *ex vivo* by histopathology. The close correlation of OCT findings with histopathological scoring demonstrates the potential for OCT as a method for non-invasive monitoring of inflammatory diseases affecting the posterior segment of the eye. Furthermore, we show that OCT is sufficiently sensitive to perform diagnostic scoring during longitudinal studies of EAU.

2. Materials and methods

2.1. Animals

Female BN rats (Charles River, Sulzfeld, Germany), 8–10 weeks of age, were used throughout the study and kept under environmentally-controlled conditions without the presence of pathogens. All experiments were approved by the local authorities of Braunschweig, Germany.

2.2. Induction of EAU

Rats were anaesthetized by inhalation of diethylether and injected intradermally at the base of the tail with a total volume of 200 μl inoculum, containing 50 μg IRBP (amino acids 521–540) (Donoso et al., 1989) in saline emulsified (1:1) with complete Freund's adjuvant (CFA) (Sigma, St. Louis, MO, USA) containing 2.5 mg/ml of *Mycobacterium tuberculosis* (Strain H37RA; Difco Laboratories, Detroit, MI, USA). Immediately afterwards, 1 μg of *Bordatella pertussis* (PTX, Sigma), (Agarwal et al., 2002) was given intraperitoneally. Controls were sham-immunized with CFA and PTX, but without IRBP.

2.3. Clinical EAU scoring

The rats were clinically evaluated daily with a slit lamp microscope, using a system detailed in Table 1A (EAU clinical stage; ECS). Rats were also weighed on a daily basis to monitor their general health.

2.4. OCT measurements

Animals were anaesthetized by intraperitoneal injection of ketamine (0.65 ml/kg; Inresa, Germany) and xylazine (0.35 ml/kg; Albrecht, Aulendorf, Germany) and placed on a platform. They were positioned below the fixed OCT probe at a 30° angle, which ensured that the incident OCT beam was perpendicular to the cornea. During alignment and centering, the eye was visualized through a digital colour-camera (Thorlabs HL-AG, Lübeck, Germany). The image acquisition was controlled with a custom-made Labview-based software (Thorlabs HL-AG), which displays the OCT image in real time and the colour-camera image of the eye. As a coupling fluid, ultrasound gel was applied both to the cornea and the OCT probe, preventing corneal dehydration and cataract formation and reducing friction between the OCT lens and the eye. Prior to imaging, pupils were dilated with tropicamide (5 mg/ml; Mydriaticum Stulln®, Pharma Stulln, Germany). Cross-sectional imaging of the retina was performed using a spectral radar OCT instrument (OCT900SR-HR, Thorlabs Inc., Newton, NJ, USA) based on the detection of optical path differences, incorporating a broadband light source with a high-speed spectrometer. Optical properties of the retinal sample were determined by analysing the back-reflected and scattered light from an illuminated retinal area. The light of a broadband low-coherence light source (centre wavelength 930 nm) was guided to the sample with a small focus to get an illuminated volume with a small lateral dimension (2 or 4 mm) leading to a good lateral resolution (2 or 4 μm). The OCT imaging engine consisted

Table 1
Scoring systems for evaluation of EAU.

A

EAU clinical stage (ECS)	Description
0	No disease
0.5	Dilated iris vessels
1	Swollen blood vessels in the iris, sporadic abnormal miosis
2	Pupil partially covered with fibrin, hazy anterior chamber
3	Exudate in anterior chamber, but pupil still visible
4	Exudate with hemorrhage (opaque anterior chamber), completely obscured pupil
5	No exudates in anterior chamber, abnormal pupil configuration, degenerating iris

Each higher grade includes the criteria of the preceding one.

B

OCT score	Description	dpi	Disease stage
0	Strong OCT signal, normal retinal morphology, no cells in vitreous	d0	Healthy
0.5	Strong OCT signal, normal retinal morphology, few cells in vitreous (mild vitritis)	d8–10	Disease onset
1	Strong OCT signal, limited FOV due to miosis, massive infiltration in the vitreous,	d12–14	Pre-peak
2	Low OCT signal, initial retinal detachment – increased RT, abnormal retinal architecture	d16	Peak
3	Very low OCT signal, coming only from choroid, limited FOV increased retinal detachment – further increase in RT, degenerating photoreceptor layer and intra-retinal areas of low reflectivity, retinal folds	d18	Post-peak
4	Blurred OCT signal due to neovascularization, increased retinal detachment – further increase in RT, abnormal retinal architecture	d20	Late disease

C

EAU histopathology score (EHS)	Description	dpi	Disease stage
0	No disease, normal retinal architecture	d0	Healthy
0.5	Mild inflammatory cell infiltration in the retina, no tissue damage	d8–10	Disease onset
1	Infiltration; retinal folds and focal retinal detachments; few small granulomas in choroid and retina	d12–14	Pre-peak
2	Moderate infiltration; retinal folds, detachments, focal photoreceptor damage; small-to-medium size granulomas, perivasculitis	d16	Peak
3	Moderate-to-marked infiltration; extensive photoreceptor damage	d18	Post-peak
4	Severe inflammation and/or full thickness retinal damage with serous exudates and subretinal bleeding, subretinal neovascularization, large granulomatous lesions	d20	Late disease

EAU clinical grading and histopatology scoring adapted from Agarwal and Caspi (2004).