

Segmenting OCT for Detecting Drug Efficacy in CRAO

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Research article

Keywords: Retinal layers, OCT, Calpain enzyme

Posted Date: August 30th, 2019

DOI: <https://doi.org/10.21203/rs.2.13754/v1>

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Version of Record: A version of this preprint was published at PLOS ONE on December 11th, 2020. See the published version at <https://doi.org/10.1371/journal.pone.0242920>.

Abstract

Thinning of the inner layers of the retina occurs in patients with central retinal artery occlusion (CRAO). The mechanism for such thinning may be partially due to proteolysis by a calcium-activated protease called calpain. Calpain inhibitor SNJ-1945 ameliorated the proteolysis in a past series of model experiments. The purposes of the present retrospective study were to: 1) use segmentation analysis of optical coherence tomography (OCT) images to mathematically model the loss of specific retinal layers in CRAO patients, and 2) predict the number of patients and days of observation needed for clinical trials of SNJ-1945 inhibitor against CRAO.

Introduction

Occlusion in the central retinal artery (CRAO) is a medical emergency due to the sudden, marked, and often permanent loss of vision in the affected eye (1). The end organ ischemia produced in retina principally affects the inner layers because the occluded central retinal artery is their main blood supply. From inside to out, the inner layers consist of the axonal retinal nerve fiber layer (RNFL), the somatic ganglion cell layer (GCL), inner plexiform layer (INPL), and the inner nuclear layer (INL). The long RNFL fibers converge as the optic nerve. The ischemia of the inner layers in CRAO is sometimes mitigated by the presence of a vessel variation, the cilioretinal artery, present in some patients. The sparing effect on loss of vision by the cilioretinal artery occurs in approximately 15% of the population and depends on size of this artery and area of the macula it supplies (2). The outer layers of eye consist of the outer plexiform layer (OPL), outer nuclear layer (ONL), outer (photoreceptor) layer, and the retinal pigment epithelium (RPE). The outer layers are generally not directly affected in CRAO because they are supplied by the separate, peripheral ciliary artery system that also supplies the choroid and iris.

Characteristics of CRAO depend on the stage. During the early acute phase, observations sometimes include swelling, retinal opacity, whitening and edema of the inner layers and optic nerve head, a cherry red spot in the macula, delayed filling of vessels in the macular arcade, arterial attenuation, box carrying of the arteries, and pyknosis of the GCL nuclei. Later stage characteristics include further vessel attenuation, atrophy and thinning of the inner retinal layers, and flattening of the foveal depression. Risk factors for CRAO are similar to systemic atherosclerosis and include ipsilateral carotid stenosis, elevated blood lipids, history of heart attack or stroke, diabetes, hypertension, smoking, and older age (2).

Treatment of CRAO is based on quick reperfusion of the retina and restoration of oxygen delivery, with an effective treatment window of within 4- 6 hours (3). Treatments include: manual ocular massage, reduction of intraocular pressure (e.g., IV mannitol), vasodilation (paper bag or carbogen inhalation), and thrombolytic therapy (tissue plasminogen activator) (4). However, there is no consensus on treatment, and the final visual acuity of 90% of non-arteritic CRAO patients without cilioretinal artery sparing is 20/400 or worse (5).

Our hypothesis is that at least part of the cellular damage to the RNFL and GCL in CRAO is due to activation of the calpain system of calcium-dependent proteases (6, 7). A calpain inhibitor, SNJ-1945, ameliorated changes in the RNFL and GCL due to hypoxia in monkey retinal transplants. This inhibitor can be administered orally and is currently undergoing human safety trials. Thus, the purposes of the present experiments were: 1) to use segmentation analysis of OCT images to mathematically describe the loss of retinal layers in CRAO patients, and 2) predict the number of patients and days of observation needed for a clinical trial of SNJ-1945 against CRAO.

Materials And Methods

Using OHSU IRB protocol # 15821, a retrospective, case control study was conducted by computer-aided search in a medical records database for CRAO (ICD10 H43.1) with at least one OCT procedure (CPT: 92134). Appropriate patients and suitable OCT reports were approved by a clinical retinologist (TSH) and an experienced ophthalmology image technician (PNS). Layer thickness values from the e2e. images were recorded for the central macula and the mean of the 4 quadrants from the 3 mm ring of the 1, 3, 6 Early Treatment Diabetic Retinopathy Study (ETDRS) grid using Heidelberg Eye Explorer License Manager Control Panel Version 3.1.0.55 (8). The two groups in this study were CRAO eyes (19 patients) and a control group made up of 15 non-CRAO eyes. Eyes were excluded on the basis of poor layer segmentation. No manual segmentation correction was performed, and repeat exams were performed in the follow-up mode. Patient data were best fit in non-linear regression analysis to a decay model:

$$\text{Layer thickness} = \text{plateau} + (\text{initial} - \text{plateau}) * e^{(-k * \text{DAYS})}$$

(k = decay constant) using Prism (GraphPad, ver.8). Tests for homoscedasticity and normality of residuals by D'Agostino and Pearson omnibus K2 were not passed. Therefore, the reference value for goodness fit, R2 (coefficient of determination), was considered biologically significant only if > 0.4. Cohen-d effect sizes were calculated as:

$$(\text{Thickness}_{\text{no drug}} - \text{Thickness}_{\text{drug}}) / \text{root mean squared error of variance (RMSE)}$$

Minimum patient numbers required per group were determined by using the Cohen-d effect size in a two tailed t-test with power of 0.8 and $\alpha = 0.05$ (9)

Results

- Demographics.

CRAO data were obtained from 19 patients with an average age at onset of 61.2 ± 16.5 years (range 31–82); 63 % were male, and 89 % were non-Hispanic white patients (Table 1). CRAO (n = 60) and 15 non-CRAO OCT scans were evaluated by segmentation software, covering a time period from 0 to 951 days from the initiation of CRAO. The number of scans per patient on different days ranged from 1 to 8.

Approximately 89 % of the patients showed severe loss of visual acuity (20/400 to detection of hand motion). Many patients showed co-morbidity factors.

- Retinal segmentation in non-affected control group.

The thickness values in the middle ETDRS ring for non-CRAO eyes (Fig. 1) were similar to literature values (10, 11) for normal patients. Except for CMT, non-CRAO layer thicknesses showed fairly low variability, even though our “normal” patients ranged in age from 31 to 82 years of age and exhibited a number of major co-morbidity eye conditions. The relative stability of the data justified using these non-affected control eyes as the “0” time point when comparing the thickness values after the initial CRAO event in Figs. 2A-C.

- Retinal layer segmentation in the CRAO group.

Affected patients in this study often showed typical signs of CRAO including, marked loss of vision, cherry red spot in macula, swelling of retina at early timepoints, attenuation of vessels, whitening of the macula, and thinning of the inner layers. Three individual patients demonstrating some of these changes are shown Figs. 2A-C. The thickness of the RNFL and GCL retinal layers decreased rapidly with time following what appeared to be an exponential decay curve (Fig. 2A, insert). Outer layer thicknesses were usually not appreciably affected by CRAO.

- Exponential decay of inner layers with time.

Changes in thickness of the retina and inner layers in CRAO patients over time fit to the basic exponential model of decay, $y = a + b \cdot e^{(-k \cdot x)}$ (Figs. 3A - F). The fit was especially robust for GCL thickness vs Days ($R^2 = 0.75$, Fig. 3D), INNER LAYERS ($R^2 = 0.69$, Fig. 3B) and the entire RETINA ($R^2 = 0.71$, Fig. 3A). The fit for RNFL was only $R^2 = 0.38$ (Fig. 3C). The INL (Fig. 3F) showed an $R^2 = 0.32$ with wide scatter, possibly due to the fact that it receives a secondary blood supply along with the central retinal artery. The outer layers (Fig. 3G-K) were not well correlated and not further analyzed.

- Patient sample size and duration of clinical trial

The specific regression formulas for each inner layer shown in each panel of Fig. 3 were used to calculate layer thickness at potential clinical trial end points of 30, 60, 90 120 and 180 days (Table 1, column A, “T0 No Drug”). Predicted decreases in thickness due to CRAO after 180 days ranged from -32 (entire retina) to -74 % (GCL) (column B).

[PLACE TABLE 1 HERE]

Sample size analysis was also performed to predict the minimum number of CRAO patients needed to detect prevention of thickness loss at various drug efficacies: 10% (Table 1, column D), 20% (column E), 30% (column F), and 50% (column G), and 75% (column H). For example, 19 patients per group (treated

and non-treated) would be required to detect 20 % efficacy in retaining the GCL layer in a 30-day trial (box). As expected, as drug efficacy increased, fewer patients would be needed (columns E, F, G & H). Composite measurements, such as the entire retina thickness (9 patients per group, 10 % efficacy, 30-day trial) and inner layer thickness (18 patients per group, 10% efficacy, 30-day trial) could also be used as more general biologic markers to follow CRAO.

Discussion

GCL thickness as a marker for following CRAO. A conclusion of the studies above was that measurement of GCL thickness would be a useful indicator of CRAO progression in a clinical trial of calpain inhibitor. Data supporting this conclusion include: 1) Previous investigators found OCT measurements in specific layers of the macula to be useful for following the progression of CRAO (12), as well as in other ocular diseases affecting the macula including age-related macular degeneration, diabetic retinopathy, retinal venous occlusion, and even glaucoma (11,13). 2) The fit ($R^2 = 0.75$) for negative exponential decay for GCL during the acute and chronic phases of our CRAO cases was good (Fig. 3A). A somewhat similar loss of total thickness in the perimacular region versus days was observed, which was described as linear in the chronic phase (14). A more recent study (15) of 134 CRAO eyes found that 78 % showed macular edema at initial baseline, and that 99 % showed retinal thinning at final examination. 3) Poor definition of the RNFL and GCL in the peri/para macular region was noted previously (16). Normal RNFL is especially thin (~ 23 μm) near the fovea, possibly causing greater error when measuring the thinner RNFL in our studies. 4) Thickness measurements for 11 layers and zones in eyes not affected by CRAO eyes (Fig. 1) were similar to those in a normative data base based on 2000 eyes (10). This validated the automated segmentation analysis used in the present study.

Establishment of clinical trial parameters. The second contribution of the current studies was that clinical trials to test a calpain inhibitor were possible with the limited supply of CRAO patients. Even with a fairly large data base, the number of CRAO patients with available OCT scans was limited, compared to more numerous central retinal vein occlusion/branch retinal vein occlusions. Using OCT GCL layer thickness monitoring, our data indicated that we could theoretically detect 20% efficacy of SNJ-1945 with 19 CRAO and 19 non-CRAO (collateral eye) patients in a 30-day trial (Table 1, column E). Stronger drug efficacies further reduced the number of required patients (columns F, G & H).

Using SNJ-1945 in such a human trial would further test our hypothesis that at least part of the inner layer thinning in CRAO is due calpain-induced proteolysis (6,17). This is based on a series of observations over the last 20 years. For example, the calpain system is present in nearly all animal tissues (7), including non-human primate (6) and human (18) retina. After central retinal artery occlusion and reperfusion in rats, retinal calcium levels increased, proteolysis was observed, and retinal ischemia caused the GCL to slough off (19). Acute ocular hypertension in rats caused decreased thickness in the IPL and INL, the number of cells in the GCL decreased, TUNEL staining in the INL increased, and a-waves in the ERGs were temporarily decreased (20) Non-human primate explants cultured under hypoxia showed calpain specific breakdown products in the RNFL followed by TUNEL-staining later in the GCL

layer. Importantly, SNJ-1945 ameliorated these changes. In cultured human retinas, hypoxia caused calpain activation and proteolysis; changes that were also ameliorated by SNJ-1945 (18). The mechanism suggested by these observations for CRAO is that occlusion causes ischemia in the RNFL, energy production by the mitochondria decreases, calcium export from the RNFL is compromised, increased intracellular calcium activates calpains, proteins such as β -spectrin in the axonal RNFL are broken down by calpains, and calpain also degrades its natural inhibitor (calpastatin). Pro-apoptotic signal activation in the axons results in apoptotic thinning of the GCL. Although less specific for this calpain/SNJ-1945 hypothesis, our data in Table 1 showed total retina thickness and composite inner layer thickness measurements could also be used as surrogate markers for CRAO.

Limitations. Patients were excluded if the segmentation errors were detected. But since only a limited number of patients with OCT scans were available, patients in this study showed a wide age range and number of OCT scans; and many co-morbidity factors, concomitant drug and treatment plans, and numerous surgeries and diseases were present. This increased the variability of the data, but represents the practical realities likely to be encountered in finding adequate CRAO patients.

Another consideration not addressed in the present study was visual acuity. 13 of the 19 patients in the present study showed marked vision loss at CF or worse. Critically, it remains to be tested if vision would be improved by partially preventing proteolysis and loss of retinal GCL and RNFL. If ischemic conditions were of significant duration or if still existed due to the no-reflow phenomenon (21), vision may not be improved by calpain inhibitor alone. Thus, a calpain inhibitor might be useful in conjunction with other treatments that quickly correct ischemic conditions in CRAO.

Declarations

Ethics approval and consent to participate: This study was reviewed and approved for ethics by the OHSU Institutional Review Board as protocol # 15821, not requiring consent for participation.

Consent for publication: NA

Trial registration: NA

Availability of data and material: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: TRS, TSH and PNS are paid consultants for Senju Pharmaceutical Co., Ltd., a company that may have a commercial interest in the results of this research and technology. MA is an employee of Senju Pharmaceutical Co., Ltd. Potential conflicts of interest was reviewed, and a management plan approved by the OHSU Conflict of Interest in Research Committee was implemented.

Funding: This research was supported in part by contract # GSODO0069A from Senju Pharmaceutical Co. to TRS.

Authors' contributions: TRS collected, analyzed and interpreted the patient data regarding CRAO. TSH vetted selection of appropriate patients. PNS optimized segmentation analysis of OCT images. MA designed the objectives and helped with interpretation of the data for the study. All authors read and approved the final manuscript.

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Acknowledgements: The authors sincerely thank Dr. Hideyuki Sakaki at the Department of Welfare and Nutrition, Kansai University of Welfare Sciences for expert advice on statistical analysis.

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Table

Due to technical limitations, the table is only available as a download in the supplemental files section

Figures

Fig. 1

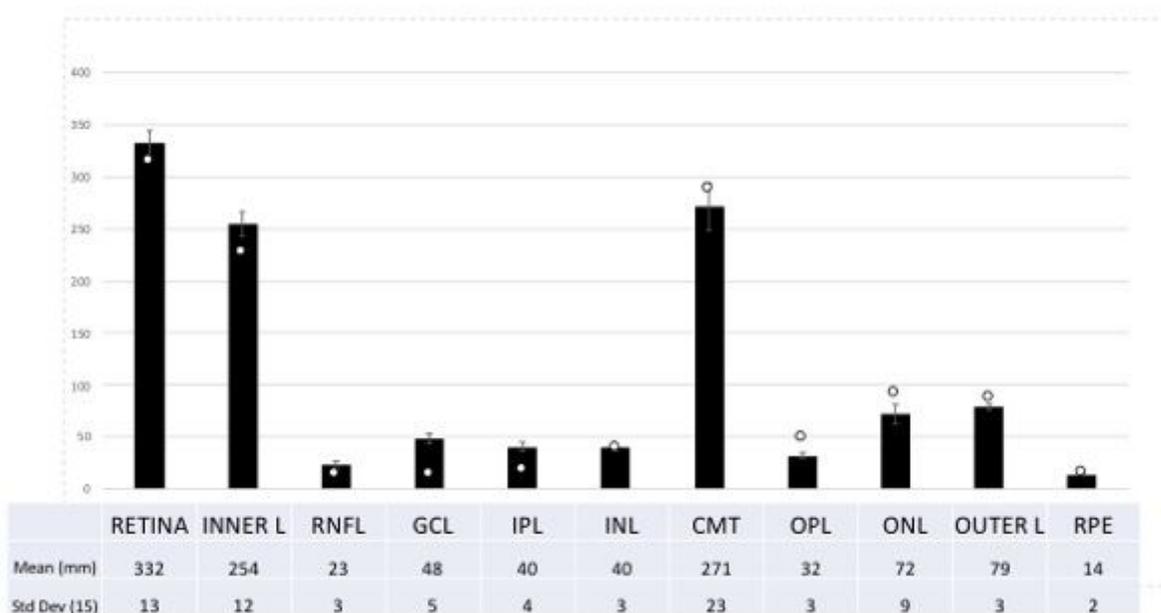


Figure 1

Non-CRAO eyes: summary of thickness values for each retinal layer with standard deviation bars from 15 patients. For comparison, the white markers on the graph show thickness values from a CRAO eye at 38 days, showing the specific effect on the inner, but not outer layers

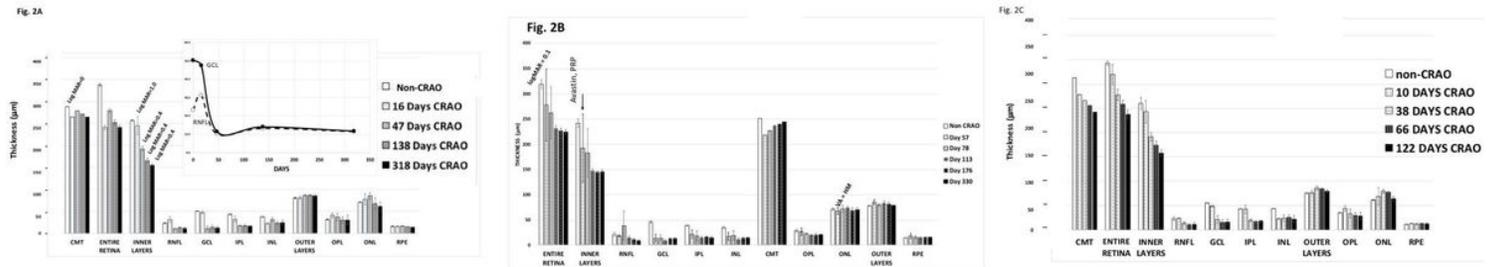


Figure 2

Mean thickness (Y axis, μm) for 11 retinal layers (x-axis) resolved by automated segmentation analysis at increasing days (increasing bar density) after the CRAO. Each figure is an individual patient showing the CRAO eye, with the contralateral eye shown as the clear bar at the far left for each layer. Error bars are \pm standard deviation for the mean thickness of the four quadrants of the middle 3 mm ETRS circle. A. Patient (partially recovered) with CRAO occurring at age 67. (Insert: Thickness changes in RNFL and GCL over time) B. Patient with CRAO occurring at age 72, with severe neo-vascular glaucoma treated with Avastin and PRP. C. Patient with CRAO occurring at age 71, with embolic stroke of undetermined origin.

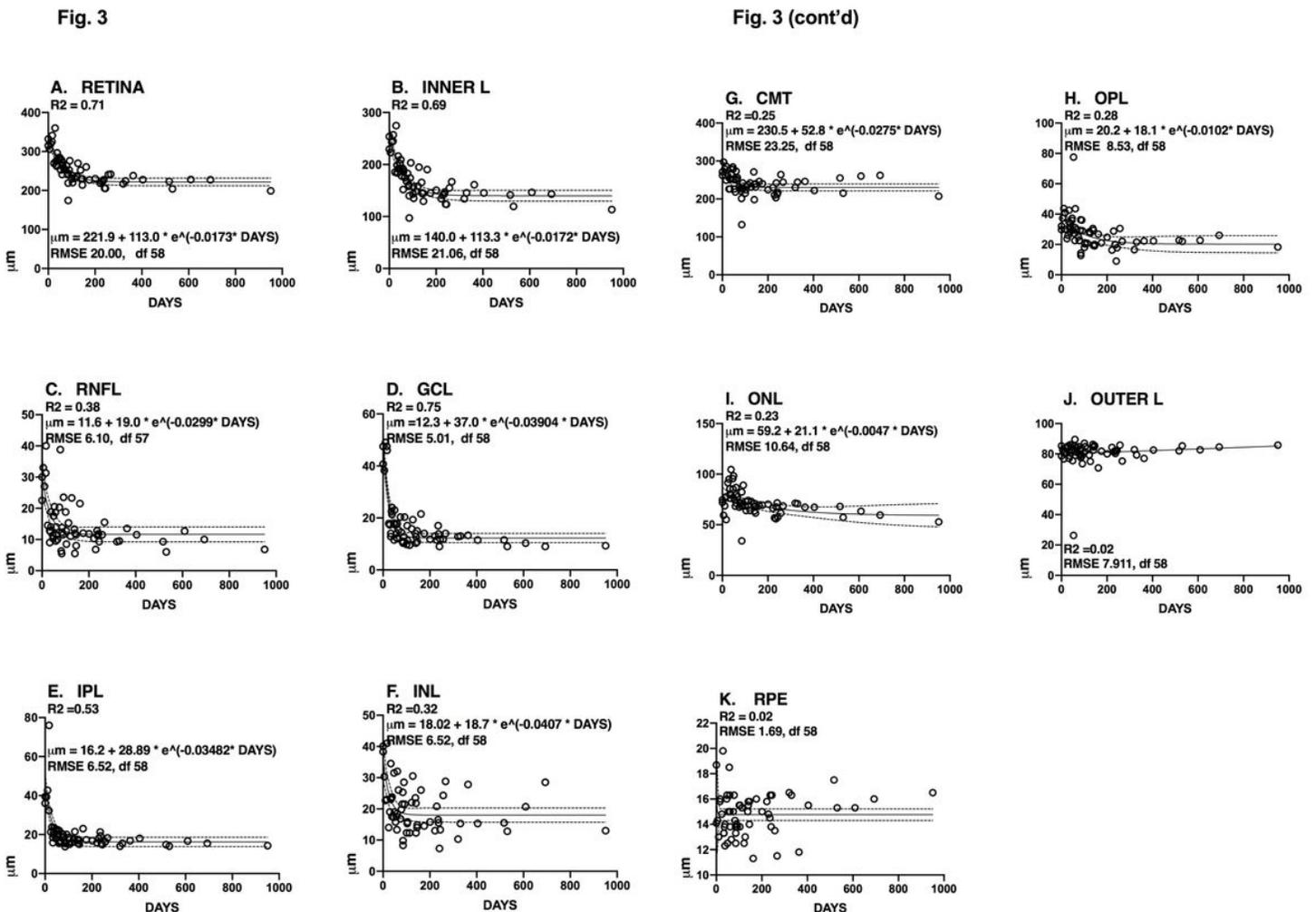


Figure 3

Regression lines (solid) and confidence intervals (dashed), showing loss of specific retinal layers during CRAO. The layer-specific exponential decay formula is shown in each panel along with the coefficient of determination (R^2). Y-axis is the layer thickness (μm). Open circles are data points from CRAO eyes at individual time points. Panels A-F include inner layers and panels G – K are the outer layers. The abbreviations used for retinal layers are: RETINA = Entire retina INNER Layers = RNFL + GCL + IPL + INL RNFL = Retinal nerve fiber layer GCL = Ganglion cell layer IPL = Inner plexiform layer INL = Inner nuclear layer CMT = Central macular thickness (average of the 1 mm diameter area in the fovea) OPL = Outer plexiform layer ONL = Outer nuclear layer OUTER Layer = Includes photoreceptor elements RPE = Retinal pigment epithelium

Supplementary Files

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- [Table1.docx](#)