**The Light-adapted Full-field ERG Luminance-response Series in Proliferative Diabetic Retinopathy Treated with Intravitreal Ranibizumab and Multispot Laser Panretinal Photocoagulation**

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# ABSTRACT

**Purpose:** To investigate the effects of intravitreal Ranibizumab (IVR), combined or not with conventional (ETDRS) or Multispot Laser Panretinal (PASCAL) photocoagulation (PRP) on light-adapted full-field ERG luminance-response series in eyes with proliferative diabetic retinopathy (PDR).

**Methods:** Laser-naive PDR patients that required PRP were randomly and prospectively assigned to receive IVR or IVR combined to PASCAL or EDTRS. PRP was performed at baseline in 1 (PASCAL) or 2 (ETDRS) sessions. In eyes with macular edema, macular laser was associated to IVR at baseline and IVR was repeated monthly, or quarterly if neovascularization was detected on angiography. Best-corrected visual acuity (BCVA), fluorescein angiography: to measure the leakage area (FLA), and OCT were performed at baseline and every 4 weeks after treatment. Full-field light-adapted electroretinography (Diagnosys LLC) was recorded using a 30 cd/m2 background and a sequence of six flashes stimuli from 0.1 to 30 cd.s/m2. B-wave amplitude data were fit with a sum of a logistic-growth and a Gaussian function (photopic hill equation). Reported parameters: VMax: sum of maximal amplitude from the two components, and μ: flash strength at the peak of the Gaussian component, in cd.s/m2.

**RESULTS:** IVR=13, PASCAL=15, and ETDRS=15 eyes finished 48-weeks follow-up. There was a significant BCVA improvement of 0.1 to 0.3 logMAR in all groups (P<0.05), and FLA reduced significantly without between-groups differences. Overall, significant a- and b-wave amplitudes reductions were observed for dark- and light-adapted ERG for ETDRS and PASCAL, but only minor dark-adapted b-wave reduction was found for IVR, up to 48 weeks. Parameter VMax reduced significantly (P<0.05) after treatment (week 12, 24 and 48 respectively) on groups ETDRS (33%, 40% and 42%) and PASCAL (34%, 30% and 33%), while a significant reduction was observed only on week 48 for IVR (13%, 13%, 22%). Parameter μ increased significantly only on groups ETDRS and PASCAL.

**Conclusions:** Parameters derived from luminance-response series were used to report cone-driven function damage on PDR eyes treated with PRP, and highlighted that IVR alone seems to avoid, at least partially, these changes. Future studies should investigate correlations between these parameters and other retinal structure/functional changes.

**Keywords:** Diabetic Retinopathy; Electroretinography; Anti-VEGF; Retinal Photocoagulation

# INTRODUCTION

Diabetic retinopathy (DR) prevalence is estimated in 30% of diabetic subjects [1]. The proliferative form of diabetic retinopathy (PDR) is characterized by the presence of pathological neovascularization, with eventual development of vitreous hemorrhages, retinal detachment and neovascular glaucoma, which are leading causes of severe vision loss in DR [2].

Established treatment of PDR consists in the use of panretinal laser photocoagulation (PRP) [3], that causes tissue destruction, but reduces retinal oxygen consuming, improving inner retina’s oxygenation [4], with consequent regression of retinal new vessels [5]. The standardized laser protocol is the recognized treatment for severe NPDR and PDR since 1991 [6].

Unfortunately, PRP can be associated with pain during application and also undesirable structural and functional retinal changes, such as macular edema [7], delayed dark-adaptation [8], visual field loss [9], and impaired color vision [10]. Thus, if possible, efforts should be made to improve or even avoid retinal photocoagulation.

More recently, another laser approach, the pattern scan laser (PASCAL), has become available [11]. PASCAL has the advantage of firing multiple laser shots at once, making the procedure less painful [12], and less time consuming [13]. Several studies compared the effectiveness of both laser strategies in the past years, and overall, conventional PRP and PASCAL showed comparable effectiveness [13], but there are reports about PASCAL being less effective than conventional PRP when looking at regression rates and prevention of neovascularization [14].

Intravitreal anti-vascular endothelial growth factor antibodies (anti-VEGF) have been used to complement PRP in the management of PDR [15]. VEGF is the major factor involved in neovascularization of PDR [16], and elevated levels of VEGF have been found in the vitreous of PDR eyes [17]. There is evidence that laser combined with anti-VEGF is more effective for PDR [18] and, of interest, the association of anti-VEGF to PRP can reduce retinal functional loss due to less extensive PRP, as we previously showed using electroretinography (ERG) [19].

The b-wave of the human photopic electroretinogram (ERG) elicited by a short flash increases in amplitude with increasing stimulus intensities at lower stimulus levels, but then decreases at higher stimulus intensities. The decrease of the b-wave amplitude at high luminance is a relatively well-known phenomenon, called “photopic hill”, and has been suggested to be related to the amplitude decrease of the d-wave at higher stimulus levels (Kondo *et al.*, 2000), and therefore intrinsically associated to the cone OFF pathway (Rufiange *et al.*, 2002), while a study in primates suggests that the origin of the “photopic hill” is related to mainly two factors: the reduction of the ON-component amplitude at higher intensities and the delay in the positive peak of the OFF-component at higher intensities (Ueno *et al.*, 2004).

In 2003, Rufiange et al described a method of analysis of the photopic hill and discuss the clinical usefulness of these parameters in cases of retinal disorders, such as CSNB (Rufiange *et al.*, 2003).

A mathematical model composed by the sum of an un-normalised Gaussian curve and a logistic growth curve, has been proposed to describe the photopic hill. This model fitted well data from normal subjects and highlighted that a CSNB1 patient shows almost no logistic component but a normal Gaussian component, further suggesting an association between the logistic growth component and on-responses and between the Gaussian component and off-responses (Hamilton *et al.*, 2007).

This model has also been recently used to document retinal dysfunction in eyes with optic nerve hypoplasia. Authors showed that if a single stimulus has been used in cases of ONH with abnormal photopic hills, the b-wave amplitudes may be either reduced or enhanced relative to adult eyes, depending on the luminance of the stimulus (Chaplin *et al.*, 2009).

In this context, the aim of this study was to describe inner-retinal function changes caused by IVR combined or not with conventional PRP or PASCAL.

# METHODS

**Ethical approval**

All procedures were in accordance with the ethical standards of the institutional research committee (Comitê de Ética em Pesquisa do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto – USP, protocol number 11685/2012) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

***Informed consent:*** All participants gave written informed consent before entering the study.

**Patient eligibility and evaluation**

A total of 45 eyes (of 34 patients) with PDR according to the guidelines of the Early Treatment Diabetic Retinopathy Study[6] were included. Further inclusion criteria were: age (18 years, or older), visual acuity better than 20/800, no earlier laser treatment, and presence of active neovascularization with immediate treatment indication. Exclusion criteria consisted of presence of intravitreal hemorrhages or tractional retinal detachment involving the macula, injection of intravitreal drugs 6 months prior to study enrollment, major opacity of cornea, crystalline lens or intraocular lens, cataract surgery 3 months prior to study enrollment, posterior vitrectomy or retinopexia with scleral introflexation, acute ocular infection, allergy to fluorescein, other ocular pathology such as glaucoma, or any medical or psychological condition at baseline examination that would not allow conclusion of study.

**Ophthalmological evaluation**

Ophthalmological evaluation was performed monthly, including assessment of LogMAR best-corrected visual acuity (BCVA), slit-lamp and fundus examination, and spectral-domain optical coherence tomography (SD-OCT – Heidelberg Engineering) to assess macular thickness. Fluorescein angiography was performed monthly to detect new vessels in the first three months, and quarterly afterwards.

**ERG protocol**

Full-field ERG was performed at baseline, and 12, 24 and 48 weeks after treatment (ColorDome and Espion E2 - Diagnosys LLC, Middleton, MA, USA). ERG was executed in accordance to ISCEV standard [20] using DTL as positive electrodes. Skin electrodes (Red-Dot – 3M) were placed on each temporal orbital rim to serve as references, and on forehead as ground. A- and b-wave amplitudes and implicit time were evaluated.

After 30 min dark adaptation, a series of flashes with increasing luminance was used as light stimuli: 0.003, 0.01 (rod ERG), 0.03, 0.1, 0.3, 1.0, 3.0 (combined rod-cone ERG) and 10 cd.s/m2. Oscillatory potentials were filtered out of combined rod-cone ERG, using an off-line fast-Fourrier algorithm set as a band-pass frequency filter (75 – 300 Hz) as previously described [21], and area under the curve (OP-AUC) between a- and b-wave implicit time was calculated.

 Thereafter, patients were light adapted for 10 min, and photopic ERG measurements were also performed a series of increasing stimuli luminance: 0.1, 0.3, 1.0, 3.0 (cone ERG), 10.0 and 30.0 cd.s/m2, followed by the 30 Hz flicker (background during photopic stimulation = 30 cd/m2).

**Group treatment assignment**

Eyes (n=45) were randomized and assigned into three different treatment groups (n=15):

* *EDTRS+IVR*: PRP with conventional single spot laser (Purepoint, Alcon, Fortworth, Texas) at two sessions (baseline and, week 2), associated with single intravitreal injection of 0.05 ml (0.5 mg) ranibizumab after first laser session;
* *PASCAL+IVR*: patient underwent PRP with multiple spot laser (PASCAL (OptiMedica, Santa Clara, California) at baseline in single session, associated with intravitreal injection of 0.05 ml (0.5 mg) ranibizumab;
* *IVR:* patient received intravitreal injection of 0.05 ml (0.5 mg) ranibizumab at baseline. In eyes with macular edema, macular shortpulse grid laser was associated to IVR at baseline. IVR was repeated monthly if central subfield thickness (CSFT) measured with spectral-domain optic coherence tomography was higher than 300 µm, or quarterly if neovascularization was detected by angiography.

After week 12, IVR was applied monthly if macular edema was detected, or every 12 weeks if neovascularization was detected.

**ERG Protocol details**

*4 stimuli of increasing luminance (4 ms white flash)*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Flash 1** | **Flash 2** | **Flash 3** | **Flash 4** |
|  | **-0.5 Log cd.s/m²** | **0.0 Log cd.s/m²** | **0.5 Log cd.s/m² [SF]** | **1 Log cd.s/m²** |
| Averages | 1 | 1 | 1 | 1 |
| Adaptation time  | **600 (s)** | 0  | 0  | 0  |
| **Acquisition** |  |  |  |  |
| Sample frequency  | 1000 (Hz) | 1000 (Hz) | 1000 (Hz) | 1000 (Hz) |
| Sweep pre-trigger time | 20 (ms) | 20 (ms) | 20 (ms) | 20 (ms) |
| Sweep post-trigger time | 150 (ms) | 150 (ms) | 150 (ms) | 150 (ms) |
| Sweeps per result | 15 | 15 | 15 | 15 |
| Interstimulus interval | 500 (ms) | 500 (ms) | 500 (ms) | 1000 (ms) |
| Drift removal | Off | Off | Off | Off |
| Manual rejection of sweeps | Off | Off | Off | Off |
| DC off-set removal | On: 20 (ms) | On: 20 (ms) | On: 20 (ms) | On: 20 (ms) |
| **Stimulus** |  |  |  |  |
| Flash duration | 4 (ms) | 4 (ms) | 4 (ms) | 4 (ms) |
| Stimulus luminance | 0.3 ph cd.s/m² | 1.0 ph cd.s/m² | 3.0 ph cd.s/m² | 10.0 ph cd.s/m² |
| Stimulus color | White 6500K | White 6500K | White 6500K | White 6500K |
| Background luminance | 30 cd/m2 | 30 cd/m2 | 30 cd/m2 | 30 cd/m2 |
| Background color | White 6500K | White 6500K | White 6500K | White 6500K |
| **Channels (1 and 2)** |  |  |  |  |
| Filter low frequency cut-off  | 0.3 (Hz) | 0.3 (Hz) | 0.3 (Hz) | 0.3 (Hz) |
| Filter high frequency cut-off  | 300 (Hz) | 300 (Hz) | 300 (Hz) | 300 (Hz) |

**Statistical analysis**

Baseline data was compared with one-way analysis of variance followed by Tukey-Kramer test for multiple mean comparisons, while group comparisons during follow-up were performed using analysis of covariance by means of a mixed-effects model, to consider intraindividual correlation, using the terms “group”, “time” and “group cross time” as effects, and a random effect was attributed to the patients’ ID followed by Tukey HSD test.

Correlations between continuous variables were investigated by calculating Pearsons’ coefficient. Calculations were performed using JMP 10.0 (SAS).

RESULTS

From the 45 eyes included, 43 (33 patients) were followed for 48 weeks: 15 eyes from group ETDRS (10 patients) and PASCAL (12 patients), and 13 eyes in IVR (11 patients). Patients’ demographic data is shown in table 1.

There were no statistically significant differences between groups regarding number of IVR injections (mean ± SE: 4.2 ± 0.2, 5.5 ± 0.5, and 4.6 ± 0.5 for ETDRS, PASCAL and IVR respectively; P = 0.1059). No difference was observed between groups regarding the frequency of diabetic macular edema detection or presence of active retinal new vessels at angiography (P>0.05).

**Best-corrected visual acuity (BCVA)**

Mean baseline BCVA (logMAR) was 0.53 ± 0.07 (20/68); 0.45 ± 0.09 (20/56); and 0.53 ± 0.11 (20/68) for ETDRS, PASCAL and IVR respectively (P = 0.7246). There was statistically significant within-group BCVA improvement of 0.1 to 0.3 logMAR in all groups during follow-up (P<0.05), without statistically significant difference between groups (P>0.05). Table 2 shows best-corrected visual acuity (BCVA, mean ± SE), best- central subfield thickness (CSFT, in µm) and fluorescein leakage area (FLA in mm2) for all groups at all study visits.

**ERG**

Overall, significant a- and b-wave amplitudes reductions were observed for dark- and light-adapted ERG for ETDRS and PASCAL, but only minor dark-adapted b-wave reduction was found for IVR, up to 48 weeks.

|  |  |
| --- | --- |
| **Baseline** | **24 months** |
|  |  |
| Figure 1: b-amplitude versus light intensity and model fit for one patient on group ETDRS at baseline and 24 months after treatment. |

Model fit has derived paramenters in all patients at all visits with acceptable fitting quality (r2 > 0.75). Figure 1 shows examples of curve fitting for baseline and 24 months follow-up in one patient from ETDRS group.

For the photopic hill-model parameters: VMax reduced significantly (P<0.05) after treatment (week 12, 24 and 48 respectively) on groups ETDRS (33%, 40% and 42%) and PASCAL (34%, 30% and 33%), while a significant reduction was observed only on week 48 for IVR (13%, 13%, 22%) (figure 2).

|  |  |  |
| --- | --- | --- |
| **ETDRS** | **IVR** | **PASCAL** |
|  |  |  |
| Figure 2: Parameter Vmax difference to baseline (µV) showing that at group IVR there was less amplitude reduction than ETDRS and PASCAL. |

Parameter μ increased significantly only on groups ETDRS and PASCAL (figure 3).

|  |  |  |
| --- | --- | --- |
| **ETDRS** | **IVR** | **PASCAL** |
|  |  |  |
| Figure 3: Parameter µ difference to baseline (µV) showing that at group IVR there no increasing stimuli intensity to reach saturation, while ETDRS and PASCAL this parameter increased approximately 0.2 cd.s/m2 after treatment. |

DISCUSSION

Many studies suggest that ERG implicit time increase are sensitive parameters to detect the functional changes in DM patients [19, 22], and as expected, it was notably changed in our cohort from the baseline on. However, they were not changed after anti-VEGF treatment, combined or not with retinal photocoagulation, and therefore were not used for group comparisons. In addition, it is also known that eyes with proliferative DR is associated with even marked ERG changes, particularly lower dark-adapted b-wave amplitude, and that retinal photocoagulation causes further ERG amplitude reduction [19].

During treatment of PDR, the goal is to inhibit angiogenesis, which is mainly controlled by expression of vascular endothelial growth factor (VEGF) that is regulated by availability of oxygen. Considering dark-adaptation as an important process causing retinal hypoxia [23], it seems reasonable to perform photocoagulation to intentionally destroy retinal structures intrinsically associated to dark-adaptation, namely, the rods.

Indeed, it has been shown that photocoagulation reduce retinal O2 consumption [4], and decrease final retinal dark-adapted sensitivity by 1.1 log units [8]. In this scenario, reductions on dark-adapted ERG amplitude are very likely and the ERG has been even suggested as an objective assessment of the degree of adequacy of panretinal photocoagulation [24].

In this perspective, as laser applications target posterior retinal structures (retinal pigment epithelium, and photoreceptors) on the peripheral retina, one could expect that ERG components generated by the posterior retina would be more affected than inner-retinal signals. However, the ERG changes found after retinal photocoagulation – slightly greater b- than a-wave amplitude reduction – might indicate that the treatment might not only destroyed the retinal areas directly illuminated by the laser beam, but also affected the functional integrity of adjacent areas, as previously hypothesized [19, 25], as far as in the macula [26]. These observations could also explain reduction on cone-driven ERG responses after PDR [19], and are certainly undesirable side-effects of the laser treatment.

Of interest, data and other reports [13] suggest that PASCAL is as effective as conventional PRP in the treatment of PDR, and it has been suggested that PASCAL laser burns cause less inner retinal destruction [25] and minor retinal sensitivity loss, with consequent only mild visual field changes detected at 6 months after treatment [26]. However, although ERG changes found on group PASCAL was slightly milder than on group ETDRS, difference between groups was not statistically significant, probably due to the small sample.

Parameters derived from luminance-response series were used to report cone-driven function damage on PDR eyes treated with PRP, and highlighted that IVR alone seems to avoid, at least partially, these changes. Future studies should investigate correlations between these parameters and other retinal structure/functional changes.

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