BLOOD-AQUEOUS BARRIER PERMEABILITY ASSESSMENT BY OCULAR FLUORESCENCE MEASUREMENTS AFTER ORAL AND IV FLUORESCEIN ADMINISTRATION

J. P. P. Domingues*, J. Figueira** C. M. Correia* and J. G. Cunha-Vaz**

* Physics Department, University of Coimbra, Portugal
** Vision Sciences Department, University of Coimbra, Portugal

jpd@ibili.uc.pt

Abstract: Optical and optoelectronics measurement techniques have been used in a clinical study to quantify ocular endogenous and exogenous ocular fluorescence in thirty individuals using a predefined protocol. Both intravenous (IV) and oral tracer administration (Sodium Fluorescein) were used and a new ocular fluorometer – Photodiode Array Fluorometer (PAF, US patent 06,013,034**) - was compared with the commercially available Fluorotron Master (FM, Ocumetrics Inc.) in this prospective approach.**

Introduction

The measurement of ocular fluorescence as a mean for physiologic functions evaluation and as a diagnostic tool began in the decade of 70 with the vitreous fluorophotometry (Cunha-Vaz et al in 1975) – a quantitative method for evaluation of Blood Ocular Barriers (BOB), mainly the Blood-Retinal Barrier (BRB). It was proven that after endovenous fluorescein administration, this substance appears in abnormal amounts in the posterior vitreous of diabetic patients with diabetic retinopathy [1][2][4]. In the eighties ocular fluorometry was pushed forward by the introduction of a commercial ocular fluorometer – the Fluorotron Master™ (FM) [2].

In order to migrate this quantitative technique to clinical practice, a new light detection and data acquisition system has been built and tested (PAF – Photodiode Array Fluorometer [3][8],). The system is based on an ophthalmic slit-lamp equipped with a solid state multi-element detector head. Driving and reading electronics is controlled by a Digital Signal Processor.

Materials and Methods

Optical setup. The PAF ocular fluorometer is to function coupled to an ordinary slit-lamp. The excitation source is the slit-lamp tungsten-halogen lamp used together with optical filters to select the appropriate wavelengths to fluorescence excitation. Fluorescence light coming from different eye structures or fluids is collected by the slit lamp objective and emission filter are there placed to prevent collection of reflected light. A beam-splitter then directs the collected light to image formation optics (spherical lens) and to a solid state linear image sensor (Hamamatsu). Cylindrical optics is also used to maximize image size and photosensitive area matching.

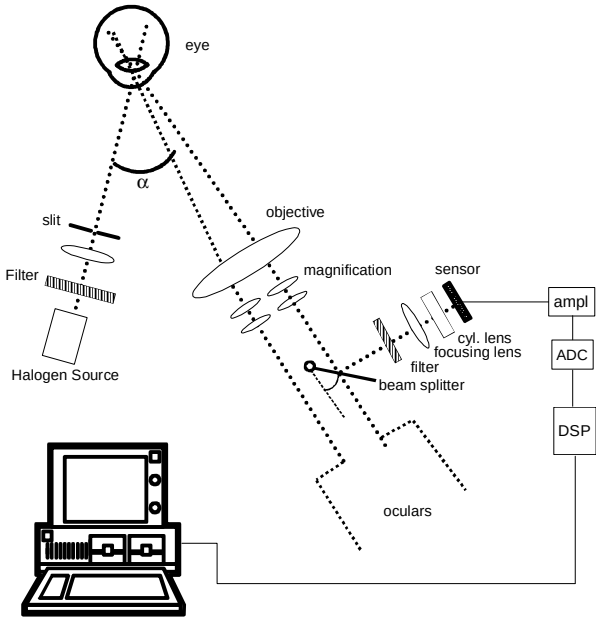

Figure 1: Overall PAF diagram

Data Acquisition and Processing. Two main electronic modules have been developed. A PC board and a detector board. The PC board has local processing capabilities provided by the Texas Instruments TMS320C25 Digital Signal Processor (DSP). From the PC point of view this is a simple memory & I/O board. A protocol has been established between PC and TMS to assure that the host can only interrupt the DSP when it is not executing important activity that cannot be interrupted. This module provides also the basic timing signals for sensor driving and for A/D conversion and all the electronics for temperature reading & control. The Interface to the sensor board is as generic as possible - 12 bit data interface and flexible timing control signals and, of course, some power lines.
The detector board, connected to the above described PC board by means of a 50 way flat cable, provides the driver/readout circuit for the sensor, the amplification

ISSN: 1727-1983

electronics to fit the sensor output to the input range of the ADC used, the ADC itself and, of course the power electronics to drive the TE cell responsible for the sensor cooling. The module is to be adapted to the ocular piece of an ordinary slit lamp that provides the optics (lenses and filters) for both excitation and fluorescence collection. A set of interchangeable excitation and emission filters can be used. The hardware/software system allows great flexibility in acquisition parameters programming and data storage and analysis and the IBM compatible environment offers good computational possibilities for graphical analysis of data.

The detector choice for this application was a self-scanning type PDA (Hamamatsu Mos Linear Image Sensor series). Among the important parameters in imaging applications of PDA's are: Sensitivity, response uniformity, dark current, pixel size (spatial resolution), quantum efficiency, saturation signal, linearity and maximum and minimum data rate. In order to evaluate detector performance by measuring some of these parameters the data acquisition system must be flexible, fast, highly programmable even for long term operation and data storage and analysis tools must be available. As we are dealing, in ocular fluorometry, with very low light levels, special care must be focused on the quality of electronics for amplification and A/D conversion. Some signal processing techniques as simple as signal averaging must also be used. The system we have developed fulfils those requirements.

Diffusion Coefficient. The rate of appearance of fluorescein in the anterior chamber after intravenous or oral administration is compared to the concentration decay of the non-protein bound fluorescein (NPBF) in plasma [3]. The diffusion coefficient is defined as the ratio from the fluorescein concentration in the anterior chamber and the time integral of NPBF in plasma.

The increase of NPBF concentration in the anterior chamber can be written as:

$$\frac{dC_a}{dt} = K_d(C_p - C_a) - K_f(C_a - C_h) - K_{a.ca}(C_a - \frac{C_c}{r_{ca}})$$

where C_a, C_p, C_h and C_c are the NPBF concentrations respectively in anterior chamber, plasma, aqueous humour and cornea. K_d is the diffusion coefficient from plasma to anterior chamber (the parameter we are interested in), K_f is the loss coefficient due to aqueous flow and $K_{a.ca}$ the aqueous to cornea diffusion coefficient. r_{ca} is simply a correction factor [3]. After integration of both sides we can approximate K_d as:

$$K_d = \frac{C_a(t_m)}{\int_{t_d}^{t_m} C_p dt}$$

Different groups from different countries reached standard protocols and methodologies for anterior segment fluorometry based on intravenous (IV) administration. For oral not much experience is available and we followed the same approach with required adaptations.

Software for automatic calculation of plasma integral by curve fitting and for calculation of diffusion coefficient in Blood – Aqueous Barrier for endovenous and oral administration has been developed.

A multicentric and prospective study was performed and thirty individuals were evaluated (10 normal volunteers and 20 diabetic patients). All of them came to the clinical center for at least two visits separated by two weeks minimum and fluorophotometry of the anterior segment using PAF and FM was performed in both eyes and all visits.

In an initial phase sodium fluorescein was intravenously (IV) administrated. On a second phase oral administration was used. Diffusion Coefficient (K_d) has been calculated for all patients in all visits. All the tests in this presentation were made using a Zeiss 30 SL/M slit-lamp with 12X optical amplification and a 5 mm high slit.

Twenty type – 2 diabetic patients were examined corresponding to 40 eyes in both centres (Instituto Galego de Oftalmologia – INGO, Santiago de Compostela, Spain and Association for Biomedical Research and Innovation on Light and Image – Associação para a Investigação Biomédica e Inovação em Luz e Imagem, AIBILI, Coimbra, Portugal). Ten were mail and ten female. Average age of 56,3 (+/- 5) years old. Mean duration of diabetis: 12,4 (+/- 5) anos. 20 eyes without Diabetic Retinopathy (DR), 18 eyes with Non-proliferative DR and 2 eyes with Pre-proliferative DR.

To ten diabetic patients sodium fluorescein was administrated intravenously in the dose of 14 mg/kg of body weight. In these patients 3 ml blood samples were collected at10, 15 and 50 minutes after injection and ocular measurements in PAF and FM were made at 30 and 60 minutes. To the other ten diabetic patients sodium fluorescein has been administrated orally after a fasting period of at least 5 hours, in a dose of 50 mg/kg using capsules of 250 and 500 mg. Blood samples were taken at 20, 30, 45, 60, 90, 120 and 150 minutes after ingestion. Ocular fluorometric measurements both in FM and PAF were made at 60 and 120 minutes after administration.

Ten normal individuals were also examined (20 eyes), five male and five female with average age of 34,5 (+/- 4) years old. These patients were only examined with oral administration of fluorescein, following the same methodology as for diabetic oral group.

In every observation a complete ofthalmologic examination with pupilar dilation using tropicamide and fenilafrine. A pre-administration ocular measurement was made both in PAF and FM.

Blood processing for plasma fluorometric measurements in PAF and FM was made according to an internationally accepted protocol [4] including centrifugation, dilution in fosfate buffer pH 7.4 and ultrafiltration for getting free, Non-Protein Bound Fluorescein (NPBF) solution.

ISSN: 1727-1983

For every patients in every visit and both in PAF and FM, two measurements were made in the same technical conditions within a five minute interval for intravisit reproducibility evaluation.

Data processing and determination of diffusion coefficient in anterior chamber. Calculations over PAF and FM fluorometric results have been made. A computer program associated to FM already exists for intravenous administration that allows automatic calculation based on existing scans. For oral administration computer program tools have been developed specifically for PAF and FM (BABo.exe). For PAF also IV tools had to be developed (BABv.exe).

Fluorometric measurements were taken in the eye (OD + OE) and in the blood plasma samples collected at precise times after fluorescein administration (a pair of measurements for each case). We also have background measurements and calibration data which allows us to convert relative ADC units into ng/ml fluorescein equivalent fluorescence units. With the developed software we calculate the concentration integrals of plasma F needed to evaluate diffusion coefficients in anterior chamber. Every patient made a second visit after a minimum period of 15 days.

The statistical methodology used was based on Spearman Rank Order Correlations test and on Wilcoxon Matched Paired test. It was considered reproducible if in Spearman Rank Order Correlations test the value of p was less then 0,05 and if in Wilcoxon Matched Paired Tests p exceeded 0,05 (no statistically significant difference). It is considered correlated if in Spearman Rank Order Correlations the value of p was less then 0,05.

Results

Both endovenous and oral examinations presented no statistically relevant deviations between FM and PAF results using Spearman Rank Order Correlations (SROC), and Wilcoxon Matched Paired Tests (WMPT) methods. For example, intravenous results of diabetic group gave $p<0.001$ (SROC).

Also good reproducibility was achieved for K_d determination in first and second visit both in PAF and FM, oral or intravenous (SROC and WMPT methods).

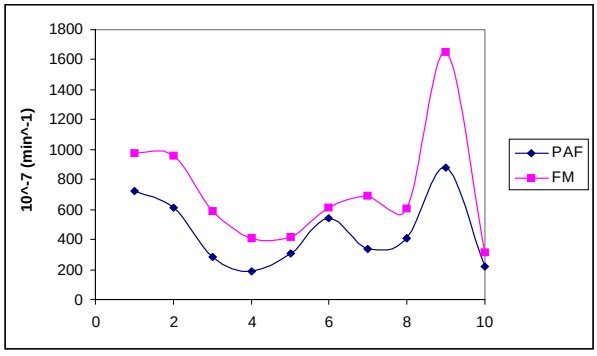

Figure 2: Endovenous K_d results for both fluorometers. X axis indicate patient number.

Reproducibility has been calculated using the expression:

$$\frac{1}{n}\sum_{n}^{1}\frac{|K_1-K_2|}{(K_1+K_2)/2}$$

where K_1 e K_2 are the K_d of first and second visit respectively. We found less then 8% for FM and less then 10% for PAF (worst cases). Intravisit reproducibility was found to be 3.6%±1.6% (IV).

1- Correlation PAF- FM (intravenous) was achieved as we have $p<0,001$ Spearman Rank Order Correlation (both eyes, 18 patients)

2- Reproducibility (endovenous – only diabetics) no significative difference between $\mathbf{K_d}$ of first and second visit (both PAF and FM) $p<0,00032$ Spearman Rank Order Correlation $p>0,748$ Wilcoxon Matched Paired Tests. Intravisit reproducibility PAF: 30': 3.3±2.4% 60': 2.9±2.5%

In vitro reproducibility: 1.2±1.1

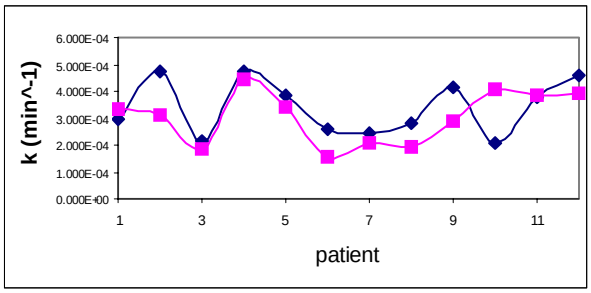

Figure 3: First (lozenge) and second (square) visit results for PAF (OD) – normal group.

Discussion

IV administration is a well established procedure and data collected gave simply the opportunity to compare measurements made with both equipments.

Taken the FM as gold standard we can then assure that the new equipment – PAF - is reliable as data collected collected from both fluorometers are correlated ($r = 0.872$; $p<0.001$). This is achieved for both visits and for both measurement times (30' and 60' in case of IV).

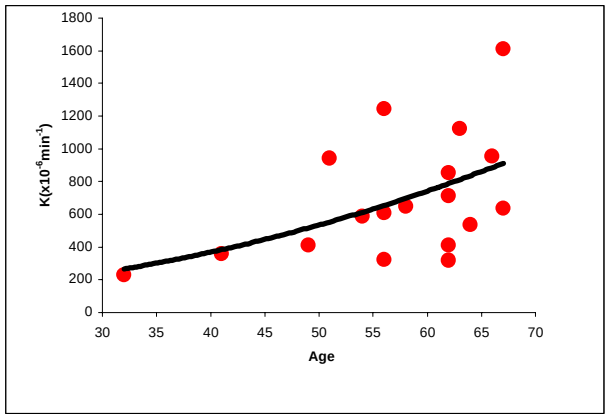

Figure 4: Anterior chamber diffusion coefficient with age.

ISSN: 1727-1983

Another objective of the study was to test a defined protocol for ocular fluorometry for oral tracer administration. Although a more complex plasma concentration profile with time, we were able to develop a curve fitting method to evaluate plasma concentration time integral.

Also attempts have been made to consider only anterior chamber concentration (either rise or wash out phases) independently of plasma measurements. Work still proceeds on that direction.

The safer oral administration together with a simplification of the procedure in order to increase patient comfort can lead this technique to a clinical routine use. Other ocular fluorometry applications like cornea and lens autofluorescence are also easily implemented with the apparatus herein described.

References

[1] JOSÉ CUNHA-VAZ, (1979): 'The Blood-Ocular Barriers', Survey of Ophthalmology, vol 23, nr 5, 1979.

[2] JOSÉ CUNHA-VAZ, (1993): 'Ocular Fluorometry: standardization and Instrumentation Development', International Opgthalmology, 17, 147-153, 1993

[3] JOSÉ CUNHA-VAZ, JOSÉ P. DOMINGUES, C. CORREIA 'Ocular Fluorometer', US Patent 06,013,034)

[4] FRANCO DOCCHIO, editor, (1997): 'Introduction to Ocular Fluorometry', European Concerted Action on Ocular Fluorometry, Coimbra, Portugal, 1997

[5] MAKOTO ARAIE ET AL., (1980): 'Aqueous Humor Dynamics in Man as Studied by Oral Fluorescein', Jpn. J. Ophthalmol. Vol 24:346-362, 1980.

[6] JAAP VAN BEST ET AL., (1996): 'Diffusion coefficient through the blood-aqueous barrier using a standard protocol.', Br. J. Ophthalmol. 80: 356-362, 1996

[7] JOSÉ P. P. DOMINGUES, CARLOS M. L. TEIXEIRA, CARLOS M. B. A. CORREIA, J. G. CUNHA-VAZ, (1997): 'The Improvement of Solid State Light Sensors Performance using Temperature Control in Ocular Fluorometry Applications', Measurement Science and Technology, 8 (Mar 1997) 322-328.

[8] JOSÉ P. P. DOMINGUES, FRANCISCO A. M. PINTO, CARLOS M. B. A. CORREIA, JOSÉ G. CUNHA-VAZ, (1996): 'Optical Characterization of a New Photodiode Array Ocular Fluorometer', Proceedings SPIE 2927, 1996 pp. 110 - 118

ISSN: 1727-1983