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Retinal microcirculation imaging in sickle cell disease patients

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ABSTRACT

Objectives: To explore the feasibility of a new quantitative method for microvascular function: non-invasive retinal function imaging (RFI). in sickle cell disease (SCD) patients and healthy controls and have it benchmarked against Laser Speckle Contrast Imaging (LSCI) measurements.
Methods: The variability of Microvascular measurements was assessed in 8 SCD patients and 8 healthy matched controls. Measurements were conducted twice on two different study days. RFI was performed for assessment of arterial and venous retinal blood flow. LSCI measurements included post occlusive reactive hyperemia and IBH challenges. Measured variables included basal flow, flow upon occlusion-reperfusion and flow during an IBH.
Results: RFI arterial flow and venous flow and LSCI basal flow and peak flow showed excellent intra subject repeatability between days (CVC of 8.5% 9.5%, 7.6% and 7.7% respectively) and between measurements on one day (CVC of 7.0%, 7.7%, 7.6% and 4.7% respectively). RFI arterial flow ($p < 0.002$), and RFI venous flow ($p = 0.007$) differed significantly between SCD patients and controls in as did LSCI basal flow, maximal flow and delta flow during IBH ($p < 0.0001$).
Conclusions: RFI showed low variability for all readout measures, comparable with most microvascular measures from LSCI. The discriminating power of the RFI between SCD patients and controls demonstrate the feasibility of this device for quantitative assessment of the microcirculation in clinical research.

1. Introduction

Sickle cell disease (SCD) is an inherited genetic disorder that affects approximately 100.000 people in the United States of America. One out of 500 African American newborns is affected by this disease and in some western European countries SCD is the most common hereditary disorder (Hassell, 2010; Elguero et al., 2015; Ingram, 1957). The disease is caused by a single amino acid substitution in the hemoglobin molecule (Ingram, 1957), which leads to a rigid, sickle-like shape of red blood cells which polymerize when deoxygenated (Bookchin et al., 1977). The polymerized sickle cells form heterocellular aggregates that alters blood flow and tissue perfusion. These acute vascular obstructions results clinically in severe painful episodes known as vaso-occlusive crisis, which often requires hospitalization and are a hallmark of SCD (Platt et al., 1994).

Hydroxyurea (HU) is the only approved drug for SCD. It increases the expression of fetal hemoglobin in the erythrocyte, which inhibits the polymerization of hemoglobin S (Platt et al., 1984). HU may also exhibit nitric oxide (NO) donor properties and induce NO-synthase activity and production in endothelial cells (Gladwin et al., 2002). Several studies show that HU reduces the occurrence of SCD-related acute complications as well as survival (Charache et al., 1995; Ferster et al., 1996; Steinberg et al., 2003; Jones et al., 2001). However, not all SCD patients respond to HU, and the exact individual factors contributing to treatment success are unclear (Ware et al., 2002). Moreover, HU therapy has side effects such as constipation, nausea, drowsiness, hair loss, and inflammation of the mouth, or even more severely neutropenia or thrombocytopenia. Hence, for some patients the risks of untreated SCD may outweigh the risks of HU's side effects (Steinberg et al., 2003). Thus, there is a need for alternative pharmacological therapies for SCD (Yawn et al., 2014).

Drug development in SCD often uses clinical measures such as vaso-occlusive crises, which are not practical for proof-of-pharmacology experiments and drug development in general. The availability of

Abbreviations: SCD, sickle cell disease; HU, hydroxyurea; NO, nitric oxide; LSCI, laser speckle contrast imaging; RFI, retinal function imaging; IBH, inspiratory breath holding; MDES, minimal detectable effect size; CV, coefficient of variation; AU, arbitrary units

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quantitative biomarkers, which report on the underlying pathophysiology leading to clinical insults, could be of great assistance. Since vaso-occlusive phenomena in SCD originate from the impact of sickled cells on the integrity and functionality of the microvasculature (Barabino et al., 2010; Epstein and Bunn, 1997; Stuart and Nagel, 2004), validated quantitative measures of microvascular function in SCD patients may provide useful pharmacodynamics biomarkers. Abnormal hemodynamics in SCD patients has been reported for several organs (Rodgers et al., 1984; Cheung et al., 2004; Cheung et al., 2002; Waltz et al., 2012; Arogundade et al., 2011; Ausavarungnirun et al., 2006; Gevers et al., 2012). Because of easy accessibility, earlier studies examined cutaneous and conjunctival microcirculation as a surrogate to investigate microvascular mechanisms in SCD. These studies demonstrated an abnormal cutaneous microvascular blood flow with periodic oscillations and a prolonged reactive hyperemic response compared to control subjects (Rodgers et al., 1984). Conjunctival red blood cell velocity is also slower in SCD patients than in control subjects (Cheung et al., 2004; Cheung et al., 2002; Gevers et al., 2012; Oguz et al., 2003).

New methodologies may allow more precise and quantitative assessments in SCD microcirculation. An interesting new non-invasive technique for quantitative assessment of microvascular function is retinal function imaging (RFI) (Ganekal, 2013; Landa and Rosen, 2010). RFI measures retinal blood flow velocity by detecting the movement of individual erythrocytes via stroboscopic illumination and high speed digital imaging of the retina. By calculating the distance that an erythrocyte moves over a series of pictures, blood velocity is quantified (Tian et al., 2016). Using RFI, early changes in retinal blood flow in diabetes (Burgansky-Eliash et al., 2010), MS patients (Jiang et al., 2016), age-related macular degeneration and retinitis pigmentosa (Beutelspacher et al., 2011; Barak et al., 2012) have been demonstrated. As such, RFI could be a valuable technique for quantification of the effects of drugs targeting endothelial integrity in sickle cell disease. However, data on retinal function in sickle cell disease patients are not available. Moreover, performance of RFI in healthy volunteers or patient populations in terms of measurement variability over day and over a longer period of time is lacking. Since these factors are crucial for rational clinical application of the technique, a series of clinical experiments was performed to assess the performance of the RFI in sickle cell disease. The variability of the measurements was assessed in SCD patients and matched controls, and retinal function was compared between moderate to severe sickle cell disease patients and healthy age-, ethnicity-, skin tone- and gender-matched controls. As reference, laser speckle contrast imaging (LSCI) (Briers et al., 1999; Roustit et al., 2010) measurements were included. LSCI measures cutaneous microvascular blood flow by detecting the movement of laser-illuminated circulating red blood cells in dermal capillaries. Although LSCI has not been used for assessment of vascular function in SCD patients before, it has been shown to be superior to laser Doppler flowmetry in terms of reduced variability and higher discriminating power when assessing microvascular function (Tew et al., 2011; Stewart et al., 2005). Laser Doppler has been commonly used in studies with SCD patients (Shi et al., 2014; L'Esperance et al., 2013). LSCI measurements included acute microvascular changes in response to a physiological challenge (post-occlusive reactive hyperemia and inspiratory breath holding).

2. Materials and methods

2.1. Study population

Variability in microvascular measures was assessed in SCD patients, aged 18–65 (n = 8), and healthy controls matched for age, ethnicity, gender, and body mass index (n = 8). SCD patients had a minimum of 4 vaso-occlusive crises in the past, and at least one vaso-occlusive crisis in the last year. SCD patients who underwent transfusion therapy within 3 weeks prior to the measurements and experienced a vaso-occlusive crisis within 1 week prior to the measurements were excluded. SCD patients continued their usual medication, including, but not limited to, hydroxyurea, vitamin D, folic acid and (prophylactic) antibiotics. For all study participants, the incidental use of acetaminophen (up to 4 g/day) was allowed.

2.2. Study design

This was an observational study, carried out at the Centre of Human Drug Research (CHDR) in Leiden, the Netherlands. Sequential RFI and LSCI were conducted twice on two study days separated by one week. On both study days, subjects arrived at the clinical unit fasted for at least 4 h. Subjects were asked to abstain from the use of alcohol from 12 h prior to each study visit, and from tobacco-or nicotine-containing products for at least 2 h prior to the each study visit, until discharge from the clinical unit. Upon arrival at the clinical unit, the subjects received a standardised breakfast. Two hours after arrival, the first block of RFI and LSCI measurements was started. The second block measurements started 2 h after start of the first measurements. All measurements were performed in climate-controlled rooms, at 20–24 °C, after a 30-min acclimatization period with the subject in supine position. During the study days, subjects were encouraged to drink sufficient water to avoid dehydration (which is especially relevant for SCD patients).

The study protocol was approved by the ethics committee of Leiden University and performed according the Dutch law on medical research.

2.3. RFI

The retinal microcirculation was quantified using the Retinal Function Imager 3005 (Optical Imaging, Rehovot, Israel). Measurements were performed as described previously (Vanzetta et al., 2014). In brief, one pupil was dilated using tropicamide. The subject remained seated quietly with the head in a headrest, which allowed collection of 10–15 series of 8 retinal images over a period of 25 min. The images were analyzed using Odian browse software (Optical Imaging, Rehovot, Israel). RFI endpoints included average arterial and average venous retinal blood flow velocity (mm/s).

2.4. LSCI

LSCI (PSI; Perimed, Järfälla, Sweden) was performed on the ventral side of the upper forearm, on a surface of 3 × 10 cm. The laser head was placed 20 cm above the skin. The frame was positioned > 5 cm from the elbow and beginning of the wrist, avoiding visible veins. A vacuum pillow was used to limit arm movements. The cutaneous blood flow was measured continuously. After the basal flow was recorded for at least 5 min, the brachial artery was occluded by inflating a pressure cuff placed around the upper arm to 200 mm Hg for 5 min. Subsequently, the cuff was deflated, inducing a hyperemic reaction. Six minutes after the occlusion, subjects were asked to hold their breath for at least 20 s. This was repeated two more times, with inspiratory breath holding (IBH) periods separated by 3 min.

Obtained data were analyzed using Perisoft software (Perimed, Järfälla, Sweden). LSCI endpoints included basal flow (arbitrary units (AU)), peak flow after occlusion (AU), ratio peak flow/basal flow (%),time to return to basal flow (seconds), post-occlusive index as previously described (Yamamoto-Suganuma and Aso, 2009) (AUC peak flow/basal flow; %), time to recovery after IBH (seconds), and the delta flow before - during IBH (arbitrary units).

2.5. Statistical analysis

For repeatedly assessed endpoints, contrasts between groups were estimated with a mixed model analysis of variance with fixed factors group (SCD versus controls), day (day 1 and day 8), measurement (0 and 2 h), group by day, group by measurement and group by day by