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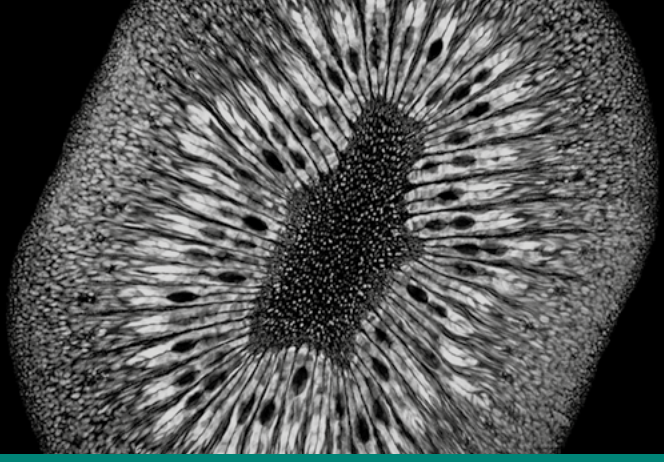

Visualization of the microcirculation

Intravital fluorescence microscopy using NiraWave™ M to monitor the microcirculation of the eye

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Introduction

The microcirculation refers to the circulation of blood in the small blood vessels within tissue vasculature, namely arterioles, capillaries, and venules, and serves several key functions within the body. In addition to its crucial role in physiological processes of tissue oxygenation and nutritional exchange, the microcirculation plays an essential role in the regulation of blood flow within tissues thereby affecting blood pressure as well as responses to inflammation[1].

In this study we use *in vivo* confocal scanning laser ophthalmoscopy (cSLO) and the innovative Viscover™ optical imaging agent, NiraWave™ M, to non-invasively monitor the microcirculation of the eye in a rat model of laser-induced choroidal neovascularization (CNV)[2]. CNV involves the abnormal growth of new blood vessels from the choroid layer into the sub-retinal space and holds great clinical significance because it is the major cause of severe visual loss. Furthermore, structural and functional changes in the microcirculation of the eye may not only be associated with eye diseases and disorders, but also with systemic pathologies that cause alterations of the microcirculation e.g. cardiovascular diseases[3]. As a result, methods that allow non-invasive visualization of the ocular microvasculature can provide useful information for disease diagnosis as well as monitoring of therapeutic interventions.

Materials and methods

To monitor structural changes in the ocular microcirculation associated with disease, experiments were performed on anaesthetized male adult Dark Agouti rats with laser-induced CNV (n = 20)[4]. Immediately after pupillary dilation using topical tropicamide (0.5 %, Mydriaticum Stulln, Pharma Stulln GmbH, Stulln, Germany) and phenylephrine eye drops (10 %, Neosynephrin-POS, Ursapharm, Saarbrücken, Germany), CNV was induced by subjecting the pupils to an argon laser (Novus 2000, Coherent, Dieburg, Germany, excitation wavelength = 514 nm, pulse duration = 0.1 s, laser power = 150 mW, spot size = 100 µm). In each eye, six laser burns were positioned concentrically around the optic nerve head.

Imaging was performed at day 7, 14 and 21 after laser treatment using a confocal scanning laser ophthalmoscope (HRA2, Heidelberg Engineering, Heidelberg, Germany) equipped with a 55° lens, operating in the near infrared reflectance mode and near infrared fluorescence mode (100 % laser power, 92 % detector sensitivity). Angiograms were obtained before and over a period of 24 h after injection of NiraWave™ M (Viscover™, *nanoPET Pharma* GmbH, Berlin, Germany) at a dose of either 0.1 mg ICG/kg body weight

(n = 5) or 0.15 mg ICG/kg body weight (n = 5), which are within the dose range recommended for clinical ophthalmic angiography (0.1 - 0.3 mg ICG/kg body weight). As a control, rats induced with CNV were injected with the clinical ICG agent (ICG-PULSION, PULSION Medical Systems AG, Munich, Germany) at the same doses (0.1 mg ICG/kg body weight (n = 5) or 0.15 mg ICG/kg body weight (n = 5)). An overall image was obtained prior to injection as well as at 2 min, 10 min, 30 min, 60 min and 24 h post injection. For quantitative analysis of fluorescence intensity, the pixel intensity was analyzed at selected regions of interest (ROI), namely in major retinal blood vessels, within laser lesions and in an area outside laser lesions, free of major retinal blood vessels (background). To prevent corneal desiccation and subsequent cataract formation as well as to optimize image clarity, lubricating eye drops (Oculotect, Novartis Pharma GmbH, Nuremberg, Germany) were regularly applied. Subsequent to imaging, Corneregel eye gel (Bausch & Lomb, Berlin, Germany) was topically applied to the eyes to promote healing.

Results and discussion

To monitor pathological alterations of the ocular micro-vasculature, rats with laser-induced CNV were injected with NiraWave M or ICG and imaged over 24 h on day 7, 14 and 21 after laser treatment. Independent of the time interval between laser treatment and imaging, strong fluorescent signals were visible in major retinal vessels immediately after injection of either imaging agent at both tested doses (0.1 and 0.15 mg ICG/kg body weight) (Fig. 1). The fluorescent signal within retinal vessels was accompanied by a weaker background signal, presumably originating from the choroidal vessels. This background signal, however, disappeared rapidly compared to the fluorescent signal within retinal vessels. At the site of laser lesions, fluorescent signals with a halo-like appearance were observed clearly after about 30 min following injection of either dye at both concentrations (Fig. 1). Of course, for each particular agent, the higher dose correlated with a higher signal strength and longer observation of the detected fluorescence. Comparison of NiraWave M with ICG at the same dose shows that the fluorescence intensity of NiraWave M in retinal vessels and laser lesions is significantly higher than that of ICG, and that its fluorescence persists for a longer period of time. In fact, at the higher dose, choroidal vessels could be distinguished for only up to 7 min with conventional ICG, whereas with NiraWave M, these vessels could be visualized for up to 28 min[4]. This is due, in part, to the increased quantum yield and higher blood half-life of NiraWave M, enabling improved imaging[5,6].

The qualitative results obtained from the eye angiograms were confirmed by quantitative analysis of fluorescent signals via pixel intensities (Fig. 2). Independent of the time interval between laser treatment and imaging, the fluorescence intensities at the three measured locations (retinal vessels, laser lesions and background) were significantly higher for NiraWave M than for ICG. Indeed, even the signal intensities obtained with the lower NiraWave M dose were stronger than those obtained with the higher dose of conventional ICG, confirming improved detection of the innovative agent by optical imaging.

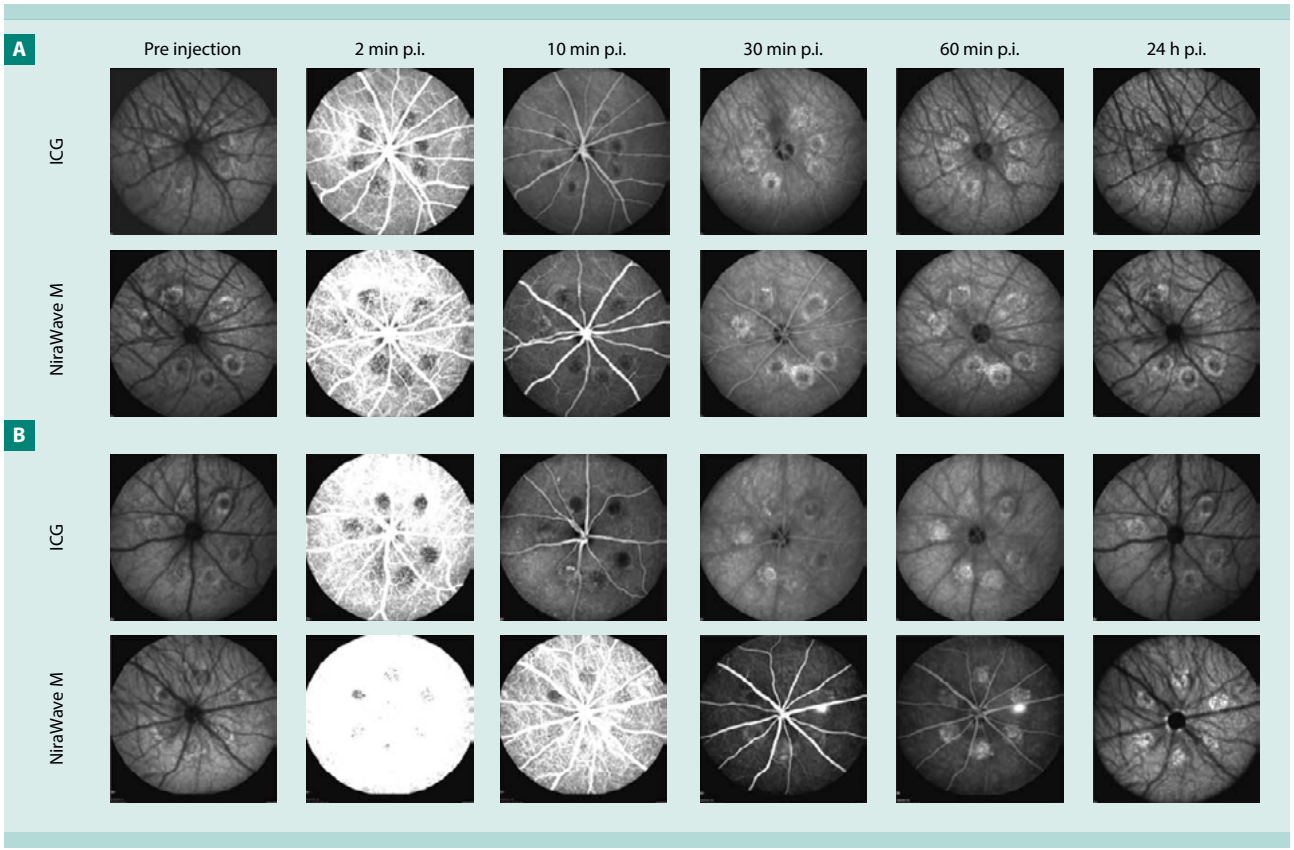

Figure 1: Eye angiograms of rats with laser-induced CNV on day 14 after laser treatment before and after injection of NiraWave M and ICG.
Images at selected doses of **A.** 0.1 mg ICG/kg body weight (lower dose) and **B.** 0.15 mg ICG/kg body weight (higher dose) show that, compared to conventional ICG, NiraWave M provides a significantly higher fluorescent signal that persists for a longer period of time.

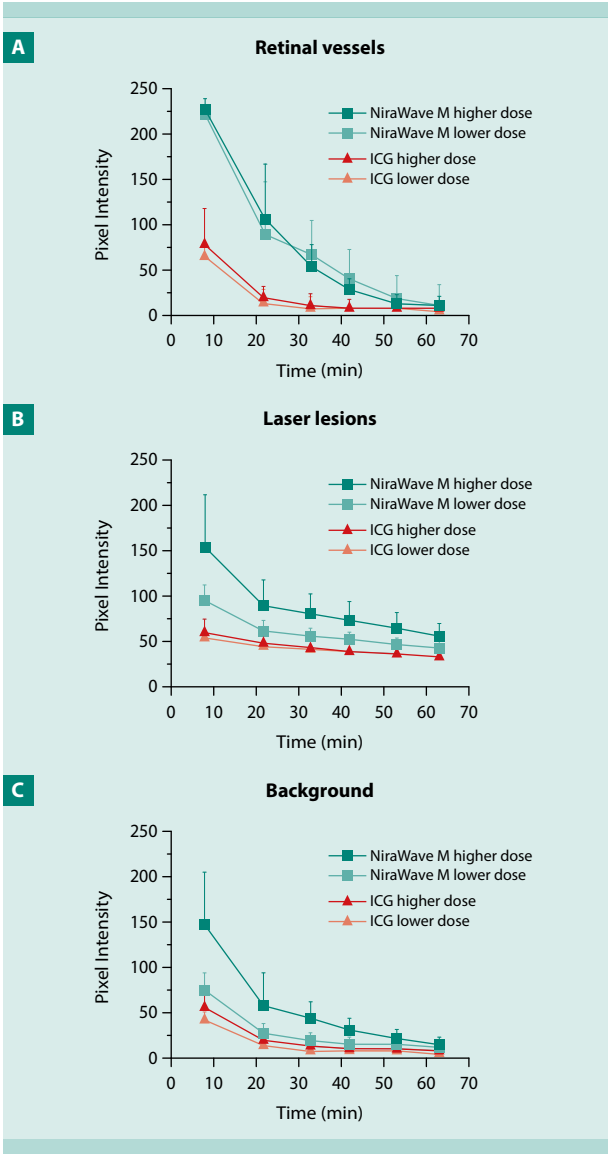

Figure 2: Quantitative analysis of fluorescent signals in the eye of rats with laser-induced CNV on day 7 after laser treatment.
Pixel intensities are shown in **A.** retinal vessels, **B.** laser lesions and **C.** background tissue after injection of NiraWave M (green) and ICG (red) at the selected doses of 0.1 mg ICG/kg body weight (lower dose) and 0.15 mg ICG/kg body weight (higher dose). Higher fluorescent signals are observed for NiraWave M regardless of the tested dose.

Conclusion

In this study we use *in vivo* confocal scanning laser ophthalmoscopy and the innovative Viscover™ optical imaging agent, NiraWave™ M, to non-invasively monitor the microcirculation of the eye in rodents. Due to its strong fluorescence intensity and prolonged blood half-life, NiraWave M is highly suited for non-invasive visualization of the ocular microvasculature, offering the possibility for improved detection and monitoring of pathological alterations of the microcirculation associated with various diseases.

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