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APPLICATION OF NIRS IN MICE: A STUDY COMPARING THE OXYGENATION OF CEREBRAL BLOOD AND MAIN TISSUE OXYGENATION OF MICE AND RAT

De Visscher Geofrey[♣♥], Verreth Wim[♣♥], Blockx Helga[♣], van Rossem Koen[♦], Holvoet Paul[♣], Flameng Willem[♣]

1. INTRODUCTION

In modern neuroscience, insight is gained in important pathophysiological processes underlying Alzheimer and Parkinson disease by the development of transgenic mice models[1-2]. Obtaining on-line information on the cerebral oxygenation, hemodynamics, cerebrovascular reactivity and mitochondrial function is crucial for the validation of these models, the understanding of the pathology itself, and the search for potential therapies. In addition, it would be very interesting to study cerebral blood flow and mitochondrial function in transgenic mice models of atherosclerosis[3] or cerebrovascular deformations leading to neurodegeneration[4].

A suitable technique for such measurement does not exist at the moment. Therefore, we tested if multi-wavelength near infrared spectroscopy (NIRS) technology, a minimally invasive technique, can be used as a tool to examine cerebral oxygenation and hemodynamics in the anaesthetised mouse.

In the first study we compare basic NIRS variables measured in mice with those obtained in rats and establish the possibility of NIRS measurement of cerebral blood and cellular oxygenation in mice. In a second study we determine the optimal dose of indocyanine green (ICG) providing a sufficiently large signal in the brain of mice and estimate the effect of the tracer injection upon the other NIRS variables

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♥ Authors contributed equally to this article
♦ Barrier Therapeutics NV, Geel, Belgium
♣ Laboratory of Cardiovascular Research, CEHA, Catholic University of Leuven, Leuven, Belgium

Address for correspondence:
Dr. De Visscher G., CEHA, KULeuven, Minderbroedersstraat 17, B3000 Leuven, Belgium,
tel: +32 16 337852 or +32 16 337298, fax: +32 16 337855

2. MATERIALS AND METHODS

2.1. Animal Preparation and Experimental Protocol

Animal housing and treatment conditions complied with the European Union directive # 86/609 on animal welfare. The study was performed on 10 male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) weighing 380-430 g, 10 C57BL6 mice (CEHA, KULeuven, Leuven, Belgium) weighing 24-26 g and 12 NMRI mice (ANIMALIUM, St. Rafael Proefdierencentrum, Leuven, Belgium) weighing 24-26 g. They were allowed free access to food and water (12/12-hour day-night cycle). The rat preparation for NIRS has previously been described in detail[5]. In short, anaesthetic induction was achieved by 4% isoflurane in 30/70% O_2/N_2O. Instead of endotracheal intubation, standard in rats, the C57BL6 mice were kept on 2% isoflurane by means of an anaesthesia mask and the NMRI mice underwent tracheotomy and were mechanically ventilated (7µl/g body weight at 150 strokes/min). In the NMRI mice a PE50 cannula was inserted into the right external jugular vein towards the vena cava for ICG injection and the aorta descendens was cannulated to monitor mean arterial blood pressure (MABP) and heart rate (HR). All animals were positioned on a rectal temperature controlled heating pad and fixed into a stereotaxic apparatus (David Kopf, Tujunga, CA, USA). The skin was removed and combined with either temporal muscle resection or retraction for rats or mice, respectively. Possible sites of bleeding were cauterised and the skull was painted black. The receiving and emitting optode, identical in both studies, were placed onto the temporal bones by means of micromanipulators. The space between the skull and optodes was filled with optical coupling gel (R.P. Cargille Laboratories Inc., Cedar Grove, New Jersey, USA) and the skull was covered with black modelling clay (Eberhard Faber GmbH., Neumarkt, Germany). Anaesthesia was then lowered to 1.5% isoflurane.

After finalisation of the animal preparation transillumination of the brain was started. The light entrance slit of the spectrograph was set at 350µm and 160µm for rat and mice, respectively. The measured variables were: concentration of deoxyhemoglobin ([Hb]), oxyhemoglobin ($[HbO_2]$), total hemoglobin ([HbT]) and oxidised cytochrome oxidase (Cu_A) as well as the optical path lengths at 740, 840 and 960nm. For the dose-finding study the same variables were measured along with MABP and HR, and in each animal, boli of 0.5, 1, 2.5, 5 and 10µl ICG were given at 5, 10, 15, 20 and 25 min after the onset of the measurements, respectively. All animals were killed by terminal anoxia.

2.2. NIRS Equipment

The NIRS system and algorithms have been developed at University College London and described in detail before[6-8]. The system allows the on-line assessment of absolute values of [Hb] and absolute changes in $[HbO_2]$ and Cu_A. Terminal anoxia at the end of an in vivo experiment allows back-calculation of the absolute $[HbO_2]$ and Cu_A. The [HbT] at each time point (= $[Hb]+[HbO_2]$) and the oxygen saturation of the hemoglobin in the cerebrum ($SmcO_2 = ([HbO_2]/([Hb]+[HbO_2]))x100$) can be calculated.

A 1mg/ml ICG (IR-125, laser grade; Acros, Geel, Belgium) solution was used containing 5% bovine serum albumin (BSA fraction V; Sigma, Bornem, Belgium) to bind the ICG. The solution was sterilized by filtration with a 0.22 µm filter unit.

NIR spectra were collected contiguously with a period of 100ms and 10 spectra were averaged to obtain a time resolution of 1 second (1 Hz) throughout the entire experiment.