Journal of Neuroscience Methods 258 (2016) 79–86

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Basic neuroscience

In vivo real time non invasive monitoring of brain penetration of chemicals with near-infrared spectroscopy: Concomitant PK/PD analysis

Francesco Crespi[a,*], Stefano Cattini[b], Maurizio Donini[b], Andrea Bandera[b], Luigi Rovati[b]

[a] Biology Dept, GSK Verona, Verona, Italy
[b] Department of Engineering Enzo Ferrari, University of Modena and Reggio Emilia, Modena, Italy

HIGHLIGHTS

- NIRS allows non invasive monitoring of brain metabolism.
- The influence of exogenous O_2 or CO_2 on the in vivo NIRS was evaluated.
- Basal HbO_2 levels in rat CNS has been attempted.
- NIRS was integrated with PK/PD analysis.
- Real time NIRS monitoring of brain penetration of chemicals was assessed.

ARTICLE INFO

Article history:
Received 18 August 2015
Received in revised form 5 October 2015
Accepted 20 October 2015
Available online 6 November 2015

Keywords:
NIRS
HbO_2
Hb
O_2
CO_2
Rat brain
In vivo
Non invasive
PK/PD
Brain penetration

ABSTRACT

Background: Near-infrared spectroscopy (NIRS) is a non-invasive technique that monitors changes in oxygenation of haemoglobin. The absorption spectra of near-infrared light differ for the oxygenation–deoxygenation states of haemoglobin (oxygenate (HbO_2) and deoxygenate (Hb), respectively) so that these two states can be directly monitored.
Comparison with existing method(s): Different methodologies report different basal values of HbO_2 and Hb absolute concentrations in brain. Here, we attempt to calculate basal HbO_2 levels in rat CNS via evaluation of the influence of exogenous oxygen or exogenous carbon dioxide on the NIRS parameters measured in vivo.
New method: Furthermore the possibility that changes of haemoglobin oxygenation in rat brain as measured by NIRS might be a useful index of brain penetration of chemical entities has been investigated. Different compounds from different chemical classes were selected on the basis of parallel ex vivo and in vivo pharmacokinetic (PK/PD) studies of brain penetration and overall pharmacokinetic profile.
Results: It appeared that NIRS might contribute to assess brain penetration of chemical entities, i.e. significant changes in NIRS signals could be related to brain exposure, conversely the lack of significant changes in relevant NIRS parameters could be indicative of low brain exposure.
Conclusions: This work is proposing a further innovation on NIRS preclinical applications i.e. a "chemical" NIRS [chNIRS] approach for determining penetration of drugs in animal brain. Therefore, chNIRS could became a non invasive methodology for studies on neurobiological processes and psychiatric diseases in preclinical but also a translational strategy from preclinical to clinical investigations.

1. Introduction

NIRS is a non invasive technique that founds itself on the use of harmless radiations, which have wavelength in the spectral range of the near infrared (650–1000 nm) (Jöbsis, 1977; Matcher and Delpy, 1993; Wahr et al., 1996). Tissues contain a variety of substances (said also chromophores) whose absorption spectrum at the wavelengths in the near infrared

* Corresponding author. Tel.: +39 0456837290.
E-mail addresses: fm.crespi@libero.it (F. Crespi), stefano.cattini@unimore.it (S. Cattini), maurizio.donini@unimore.it (M. Donini), andrea.bandera@unimore.it (A. Bandera), luigi.rovati@unimore.it (L. Rovati).

http://dx.doi.org/10.1016/j.jneumeth.2015.10.011
0165-0270/

region are known and present in such quantity to allow to find their concentrations through measures of attenuation. The concentration of some chromophores as water and melanin, are constant during the time of measure, while some mixtures as HbO_2 and Hb are characterized by concentrations tightly correlated to the level of oxygenation and the metabolism of tissues. Furthermore, total haemoglobin concentration (HbO_2 + Hb) is considered as total blood volume (*V*) (Chia-Wei and Ching-Cheng, 2012).

Generally, main applications of NIRS is in the study of the transport of the oxygen to the muscles, the tissue oxygenation index, the cellular metabolism and the cerebral haemodinamics. In clinical studies NIRS is currently employed to monitor fetal hypoxaemia (Aldrich et al., 1994) and in newborn infants to detect birth asphyxia and/or apnoea and hypoxia (Brazy et al., 1985; Wyatt et al., 1986; Meek et al., 1999).

In addition, changes in oxygenation during occlusion of the carotid artery, or during cardiopulmonary bypass, circulatory alterations and/or arrest are also analyzed using NIRS (Quaresima and Ferrari, 1998; Pogue et al., 2001).

Besides fewer NIRS studies have been performed at the Central Nervous System (CNS) level, mainly to monitor oxygen sufficiency and brain functions and/or brain mapping (Villringer and Chance, 1997; Obrig et al., 1996; Colier et al., 1999) as well as brain diseases (Hock et al., 1996; Suto et al., 2004; Kubota et al., 2005) while only a limited amount of studies have used NIRS to analyse "vascular CNS" functions in animals. Therefore, in order to implement such type of analysis, we have developed a Near Infrared Continuous Wave Spectroscopy instrument (NIR-CWS, based on the low extinction coefficient of tissue in the near infrared region, Rovati et al., 2003) that allows in vivo, real time non-invasive NIRS measurements in the rat brain (Crespi et al., 2005; Crespi, 2007). In particular, in this study an attempt to calculate basal HbO_2 levels in rat CNS has been attempted via evaluation of the influence of exogenous oxygen or exogenous carbon dioxide (CO_2) on the NIRS parameters measured in vivo. Furthermore the possibility that changes of haemoglobin oxygenation in rat brain as measured by NIRS might be a useful index of brain penetration of new chemical entities has been investigated. To test this hypothesis, different compounds from different chemical classes were selected on the basis of parallel ex vivo and in vivo pharmacokinetic (PK/PD) studies of brain penetration and overall pharmacokinetic profile. In particular, two NK1-SSRI receptor antagonists having very similar chemical/molecular characteristics [GSK communication] as well as two glycine-1 transporter inhibitors were selected based on preliminary ex vivo pharmacokinetic studies showing either a high or low brain penetration (B/B) ratio, respectively. The respective effects of these chemical entities on brain metabolism were then assessed using NIRS in anaesthetised rats.

Successively, parallel in vivo pharmacokinetic/pharmacodynamic and NIRS experiments have been performed using a further compound: GSK18106 a glycine receptor antagonist known to have high brain penetration (B/B) ratio [i.e. 1.8 at 5 min; 2.1 at 15 min; 4.5 at 30 min; 3.2 at 60 min, respectively, GSK communication].

2. Material and methods

2.1. Chemicals

Two NK1-SSRI receptor antagonists (GSK13025 and GSK18111); two glycine-1 transporter inhibitors (GSK270694 and GSK26870) and the glycine receptor antagonist GSK189106 [all GSK compounds] were utilized in this study.

Table 1
Ex vivo PK/PD data: brain exposure 1 h post treatment: 1 mg/kg (n = 4 each treatment).

NK1-SSRI receptor antagonists		Glycine-1 transporter inhibitors	
GSK13025	106 ± 14 ng/g	GSK27694	388 ± 89 ng/g
GSK18111	25 ± 5 ng/g	GSK26870	13 ± 5 ng/g

2.2. Standard liquid chromatography–mass spectrometry (LCMSMS) analysis

These analysis have been performed as described (Marzo and Dal Bo, 2007) and brain exposure [ng/g] as well as brain penetration (B/B) ratio of the compounds tested are presented in Table 1, and in Section 3.

2.3. NIRS studies

In the present work we have used the Near Infrared Continuous Wave Spectroscopy instrument (NIRCWS,based on the low extinction coefficient of tissue in the near infrared region) that allows in vivo, real time non invasive NIRS measurements in rat brain as demonstrated earlier (Rovati et al., 2003; Crespi et al., 2005; Crespi, 2007). In particular, it allows performing quantitative assessments of haemoglobin variations exploiting precise absorption measurements close to the absorption peak of the water, i.e. 975 nm (Rovati et al., 2003). Under these conditions, the dominant absorbers are haemoglobin and water. They contribute to determine the value of absorption coefficient i.e. the log 10 of the ratio of impinging and back reflected intensity per unit of the sourcereceiver distance (unit OD $\measuredangle$ cm^{-1}) (Schmidt, 1999).

During the whole in vivo experiments, the optical probes are positioned close to the intact surface of the animal head and covered with a black tissue for better coupling and isolation from ambient light. Moreover, the NIRCWS instrument implemented here has been set to reject continuous as well as alternate ambient light by means of a particular "time-variant" shaping of the NIRS signal (see Rovati et al., 2003).

Prior to the entrance into the brain, NIR light is passing through hair, skin and skull (bone), therefore the influence of such "layers" on HbO_2, Hb and Volume (HbO_2 + Hb) signals has been analysed as already described (Crespi et al., 2005). Briefly, in anaesthetised (Ketamine 0.8 ml/kg i.m. + Xilazine 0.5 ml/kg i.m.) adult male rats were held in a stereotaxic frame and a set of 4 optic fibres (200 μm diameter) and a receiver were positioned upon the sagittal line as described previously (Crespi et al., 2005, 2006). In particular, here two optical fibres were set directly in contact with the *dura madre* via a drilled hole in the parietal bone, while the other two optical probes were in contact with the intact surface of the rat's head (Fig. 1). Three intact rats and three "drilled" rats were used and treated with vehicle (NaCl 0.9%) and d-amphetamine (2 mg/kg), sub-cute (s.c.) after a 10 min period of control recordings. The measurements continued for an additional period of 30–40 min.

In all the successive experiments only intact rats were studied. In vivo non-invasive real time NIRS measurements of HbO_2 and Hb were then performed at first to analyse the influence of exogenous Oxygen (O_2) or exogenous carbon dioxide (CO_2) on the NIRS parameters measured in vivo.

For this experimental measurement two different kinds of alternate breathing have been used in six animals: one of pure oxygen and one of normal air. This allows reaching two different percentages of inspired oxygen, equal to a 20% (normal air) and a 100% (pure oxygen). Then exogenous CO_2 is supplied as it is done for O_2 i.e. by means of a canula inserted into the mouth of the anaesthetised rat.