MONITORING C EREBRAL OXYGENATION *IN VIVO* BY REFLECTANCE SPECTROSCOPY

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Abstract. In this work, the overall results on the feasibility of realizing *in vivo* local oxygenation measurements using a spectroscopic technique are reported. In order to achieve *in vivo,* local monitoring of hemoglobin oxygen saturation, optical fiber probes were inserted into the rat striatum. The probes consisted of two optical multimode fibers stack together. One fiber was used to illuminate the medium by means of a halogen stabilized white lamp while the other one was used to collect the reflected light and direct it to a grating spectrometer equipped with a cooled CCD camera. By measuring the reflectance spectra the chromophores concentrations and hemoglobin oxygen saturation were calculated during different experimental protocols while progressive hypoxia episodes were performed by artificial ventilation with different mixtures of oxygen and nitrogen. Our results proved that our technique provides feasible results on the temporal variations of some physiological parameters such as hemoglobin and sufficiently sensitive to distinguish various hemodynamic effects such as tracking an optical bolus, to detect a cerebral ischemia and to observe effects such as variations in the redox state of the respiratory enzymes.

Key words: hemoglobin, hypoxia, brain, oxygen saturation, spectroscopy, optical fibers.

INTRODUCTION

Near infrared spectroscopy (NIRS) is now being used in clinical diagnosis as a non-invasive monitor of the tissue oxygenation. However, because near infrared light is less absorbed by the biological tissues than visible light, the near infrared spectral window is suited for non-invasively global brain oxygenation studies. Unfortunately, the interpretation of NIRS data is controversial because the specific absorption spectra of oxygenated hemoglobin (HbO_2), deoxygenated hemoglobin (Hb) and oxidized cytochromes (CtOx) overlap and are relatively featureless [6, 10].

Furthermore, the tissue absorption coefficients caused by hemoglobin are an order of magnitude greater than those caused by oxidized cytochrome hence an important cross talk between the hemoglobin and cytochrome signals. To

Received July 2005;
in final form October 2005.

overcome these limitations we developed a prototype based on reflectance spectroscopy of the visible light as a technique to asses *in vivo*, locally, the cerebral oxygenation status.

MATERIALS AND METHODS

SPECTROSCOPIC SET-UP

In order to assess *in vivo,* local monitoring of hemoglobin oxygen saturation, optical fiber probes were inserted into the rat striatum. The probes consisted of two optical multimode fibers of 100 μm diameter stack together. One fiber was used to illuminate the medium by means of a halogen stabilized white lamp while the other one was used to collect the reflected light and direct it to a grating spectrometer (Princeton Instruments, New Jersey, USA) equipped with a cooled high dynamic range CCD camera (Princeton Instruments, New Jersey, USA). Both the spectrometer and the CCD camera were driven by a computer with Winspec software (Roper Scientific, New Jersey, USA). Due to the small separation between the emitting and collecting fibers and to the important scattering coefficient of the cerebral tissue, we have not been in a diffusive regime [8].

This is why we had to perform Monte Carlo simulations in order to achieve information on the spatially volume explored by the light. Our simulations showed that this volume is inferior to 1 mm. In simulations, a non absorbing optical phantom made of a suspension of polystyrene spheres was considered. The scattering coefficient and the anisotropy factor characterizing this medium were found by means of a Mie calculation (Mie scattering on spherical particles) in which the index of refraction of the polystyrene spheres was 1.55, their diameter 435 nm and their volumic concentration 0.24%.

The reason of using an optical phantom made of polystyrene suspensions was that they are very easy to be maneuvered, as technically is not so difficult to set their dimension, and so to prepare media characterized by various optical properties. Moreover, experimental studies [4], have proved their optical properties stability.

By means of such prototype instrument, reflectance spectra within the spectral range 500–620 nm were recorded. Spectra were acquired with a 1s sampling time and averaged over 1 minute.

ANALYSIS OF DATA

The reflectance spectrum was obtained according to the relationship:

$$R(\lambda_i) = \frac{I_r(\lambda_i) - I_0(\lambda_i)}{I(\lambda_i) - I_0(\lambda_i)} \tag{1}$$

where $I_r(\lambda_i)$ was the reference spectrum measured with the optical probe immersed into a scattering medium modeling the non absorbing cerebral tissue (suspension of polystyrene micro spheres of 435±30 nm diameter in distilled water; the polystyrene concentration was 92%), $I_0(\lambda_i)$ was a baseline spectrum due to the artefacts of the probe immersed in distilled water while $I(\lambda_i)$ was the *in vivo* spectrum measured when the probe was inserted into the rat brain.

The chromophores concentrations were extracted by fitting the Beer-Lambert equation:

$$\log R(\lambda_i, t) = [\varepsilon_{\mathrm{HbO_2}}(\lambda_i)c_{\mathrm{HbO_2}}(t) + \varepsilon_{\mathrm{Hb}}(\lambda_i)c_{\mathrm{Hb}}(t) + \varepsilon_{\mathrm{EB}}(\lambda_i)c_{\mathrm{EB}}(t)] \times K + B \quad (2)$$

using a singular decomposition fitting method [9].

In the previous equation, $\varepsilon_{\mathrm{HbO_2}}(\lambda_i)$, $\varepsilon_{\mathrm{Hb}}(\lambda_i)$ and $\varepsilon_{\mathrm{EB}}(\lambda_i)$ stand for the specific absorption coefficients of oxygenated hemoglobin, deoxygenated hemoglobin and of an eventual dye and $c_{\mathrm{HbO_2}}(t)$, $c_{\mathrm{Hb}}(t)$ and $c_{\mathrm{EB}}(t)$ stand for their tissue concentrations measured at the instant t, respectively.

K is a constant taking into account the path length of the light in the tissue and the difference between the scattering properties of the optical phantom and cerebral tissue B was a baseline.

Thus, the hemoglobin cerebral oxygen saturation could be calculated according to the formula [1, 2]:

$$ScO_2(t) = \frac{c_{\mathrm{HbO_2}}(t)K}{c_{\mathrm{HbO_2}}(t)K + c_{\mathrm{Hb}}(t)K} \times 100 \quad (3)$$

From this equation we can conclude that practically there is no need to know the parameter K value.

In fact, earlier we showed [1] which are the experimental conditions, which have to be fulfilled in order to have K nondependent on wavelength. We have no explanation for using the baseline B into the fitting procedure. We simply observed that the experimental data are very well fitted by the theoretical curve when it is used.

ANIMALS AND EXPERIMENTAL PROTOCOL

Adult, female rats were anesthetized at the beginning of the experimental protocol with isoflurane. Cannulas were inserted into a femoral artery for blood pressure monitoring, arterial blood sampling and continuous thiopental injection (1–2 ml/h in order to maintain anesthesia during experiments) and into a femoral vein for dye and physiological serum administration.

The blood samples allowed us to monitor continuously some physiological parameters such as the oxygen arterial partial pressure and CO_2 arterial partial pressure. Rectal temperature was also monitored by a thermocouple and maintained at 37 °C by means of a feedback controlled hot water pad.

Progressive hypoxia was induced by a stepwise changing of the fraction in inspired oxygen (FiO_2). Thus, each experimental protocol involved 6 hypoxic episodes corresponding to different values of FiO_2: control (FiO_2 of 35%), normoxia (FiO_2 of 21%), hypoxia (FiO_2 of 15%, 12% and 10%), reoxygenation (FiO_2 of 35%) and anoxia (FiO_2 lowered from 35% to 0%).

The control period was necessary for the hemodynamical stabilisation of the animal after tracheostomy, and the reoxygenation episode was performed by restoring the oxygen in the gas mixture. Excluding the anoxia episode, all others episodes lasted for 15 minutes.

RESULTS AND DISCUSSIONS

Statistical results on the evolution of the ScO_2 obtained by averaging the ScO_2 values over 10 rats being shown in figure 1. These results can be analyzed in three manners: coherence of the ScO_2 values obtained during the experimental protocol with the values of the fraction of inspired oxygen, correlation of the ScO_2 values with simultaneous cerebral blood flow measurements found by Doppler velocimetry and comparison of the ScO_2 values with the arterial (SaO_2) and venous (SvO_2) oxygen saturation issued from blood samples.

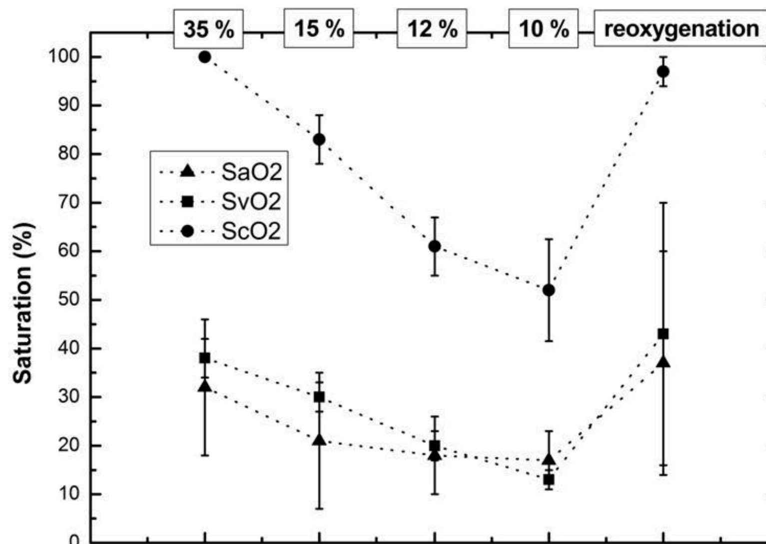

Fig. 1. Evolutions of the arterial (SaO_2) and venous (SvO_2) oxygen saturation issued from arterial and venous blood gas analysis and ScO_2 found by reflectance spectroscopy, *in vivo*. The results are averaged over 10 animals.

CEREBRAL OXYGEN SATURATION VS FRACTION IN INSPIRED OXYGEN

Qualitatively, the ScO_2 evolution with FiO_2 was coherent, the oxygenation levels corresponding to different FiO_2 values being very clear. Nevertheless, it has to be noted that the mixing gas system allowing to control the FiO_2 values was not able to achieve feasible values of the FiO_2 between the oxygenation levels, so that one cannot make a continue comparison of the ScO_2 values with those of the FiO_2.

Quantitatively, the results are not feasible always. Two negative aspects have to be mentioned: ScO_2 values were too low during the control and normoxia episodes and a large variation of the results from an animal to another. It is true that the number of variables, which can influence our results, were large. In fact, the measurements *in vivo* could be influenced by the hematocrit variation in the explored area. Though in our studies the hematocrit variations have been maintained at their physiological limits during hypoxia, the hematocrit can change from an animal to another and so, our results could be explained.

Furthermore, apart from the hematocrit issue, the presence of the large blood vessel at the proximity of the optical probe could have important effects on the acquired optical signal. Finally, the fitting of the spectra was precise for the spectra recorded during the episodes poor in inspired oxygen. This helplessness of the mathematical algorithm to fit data could be explained by the fact that the scattering properties of the cerebral tissue change when the tissue itself is poor in oxygen. This is an effect already observed by several researchers [3, 5, 7] but at the moment there is no plausible explanation for it.

CEREBRAL VS ARTERIAL AND VENOUS OXYGEN SATURATION

The gas blood analysis allows extracting the values of the arterial and venous oxygen saturation. Even though the number of blood samples authorized by the biological protocol does not allow having a continuous comparison SaO_2-SvO_2-ScO_2 however, even if one analyze only a few data one can reach some important conclusions. Thus, the experimental data given in figure 1 show that the ScO_2 values are closer to those of SvO_2 values, though a variability of the data is more important for ScO_2. That means that the ScO_2 values reflect the oxygen saturation of the venous compartments, conclusion that maybe pertinent if we take into account the fact that the brain is occupied by the venous compartment in a proportion of about 70%. Nevertheless, the comparison SaO_2-SvO_2-ScO_2 has to be done knowing that ScO_2 represents the oxygen saturation of the cerebral tissue locally measured while SaO_2 is a systemic quantity and SvO_2 is a quantity averaged throughout the brain. Hence, figure 1 can be analyzed only qualitatively: changes in ScO_2 reflect primarily those in brain venous oxygenation.

These statistical results show that the temporal evolution of the ScO_2 values during the experimental protocol is coherent with the SaO_2 and SvO_2 temporal evolutions. As the explored volume changed from an animal to another, we can say that, without doubt the SvO_2 values are given by the specific local arteriovenous distribution.

CEREBRAL OXYGEN SATURATION VS CEREBRAL BLOOD FLOW

Measurements of the cerebral blood flow (CBF) were performed at the same time with the spectroscopic acquisition by inserting into the same cannula of a Doppler probe in such a way that the explored volume by both optical and Doppler probes was approximately the same. It could be seen that the CBF evolution is almost a reflection in a mirror of ScO_2. It is a confirmation that with our prototype one can measure at least changes of ScO_2. Nevertheless, one could observe a quicker lowering of ScO_2 than the increasing of CBF when FiO_2 varies from 21% to 15%. This effect could be due to some physiological factors. Thus, the quicker decreasing of ScO_2 while CBF augments corresponds to a lower oxygenation of the blood inducted by hypoxia without any modification of the oxygen extracted by the tissue.

This physiological phenomenon observed by many researchers was attributed to the fact that the increase of the CBF intervenes from an oxygenation threshold corresponding to a level of about 55 mmHg of the oxygen arterial partial pressure. In our experimentations, the level of the arterial oxygen partial pressure was 66.7±7.8 mmHg for a FiO_2 of 21% and 45.6±3.1 mmHg for a FiO_2 of 15%.

CONCLUSIONS

The first thing which has to be remarked is that fitting data using equation (2) gave us very good adjustments of the experimental data. Hence the conclusion that the experimental and theoretical methods and methodologies used in building our prototype were correctly chosen and used. As positive aspects it has to be evidenced the capacity of the system to measure ScO_2 accurately in such a way that the ScO_2 can very clearly follow the FiO_2 values, one can monitor certain respiratory enzymes, detect a cerebral ischemia, etc.

Furthermore, flow blood measurements by Doppler velocimetry proved the capacity of the system to be used in monitoring temporal variations of the chromophores concentrations and temporal variations of ScO_2. As negative parts of our analyses we have to remark some randomly inexact fittings (especially during episodes when the tissue was poor oxygenated) and some false value for the ScO_2 even if the fit was correct.

Of course there are many things witch can explain all the negatives aspects: possible haemorrhages, movements of the optical probes, the fact that the cerebral tissue has not exactly the same optical properties as the reference medium, an important variation of scattering coefficient with the wavelength and finally the inhomogeneities of the biological tissue.

Altogether, even in its actual state of development, the proposed prototype can be used for invasive explorations of the deep cerebral tissues being complementary and compatible with the NMR technique, offering portability and a price, which make it easily utilizable in various scientific environments.

Even if the method we used is clearly invasive, it is clinically accepted. The main advantage of our technique over the non invasive ones is that the tissue oxygenation monitoring is done *in vivo*, locally. None of the other techniques can offer such information.

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