Modelling blood flow and metabolism in the preclinical neonatal brain during physiological insults

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1. Introduction

Neonatal brain injury can result from hypoxic-ischaemia - reduced oxygen delivery and/or blood flow. This can occur due to complications either in the uterus or during delivery, often causing permanent damage to the patient's brain. The natural physiological response of the brain in such instances is complex and not well understood.

Piglets are often used as models of human neonates for clinical experiments involving anoxic and/or hypoxic and ischaemic insults. The purpose of this work is to increase understanding of the metabolic and circulatory processes occurring during anoxic, hypoxic and ischaemic insults in piglets. Therefore, we have created a computational model of blood flow and metabolism in the neonatal piglet brain (BrainPiglet) to investigate the effect of such insults [1]; this model is an adaptation and extension of an earlier model of the adult human brain [2]. The model is able to simulate data from near-infrared spectroscopy (NIRS) and magnetic resonance spectroscopy (MRS) – two non-invasive measurement methods that are used to monitor brain tissue oxygenation, haemodynamics and metabolism. The model has been shown to successfully simulate NIRS and MRS data during brief anoxias in piglets, and predict in 1-day old piglets the brain's underdeveloped capacity to autoregulate [1]. We have recently expanded the BrainPiglet model further, to model brain hypoxia-ischaemia (HI) by simulating carotid artery occlusion and changes in cellular pH. Our aim is to use the extended BrainPiglet model (v2) to integrate our measurements and decipher the precise haemodynamic and metabolic events in the early aftermath of perinatal HI. Our goal is to understand how these early physiological and metabolic changes relate to subsequent injury severity, and help provide vital clues to injury pathways and neuroprotective strategies.

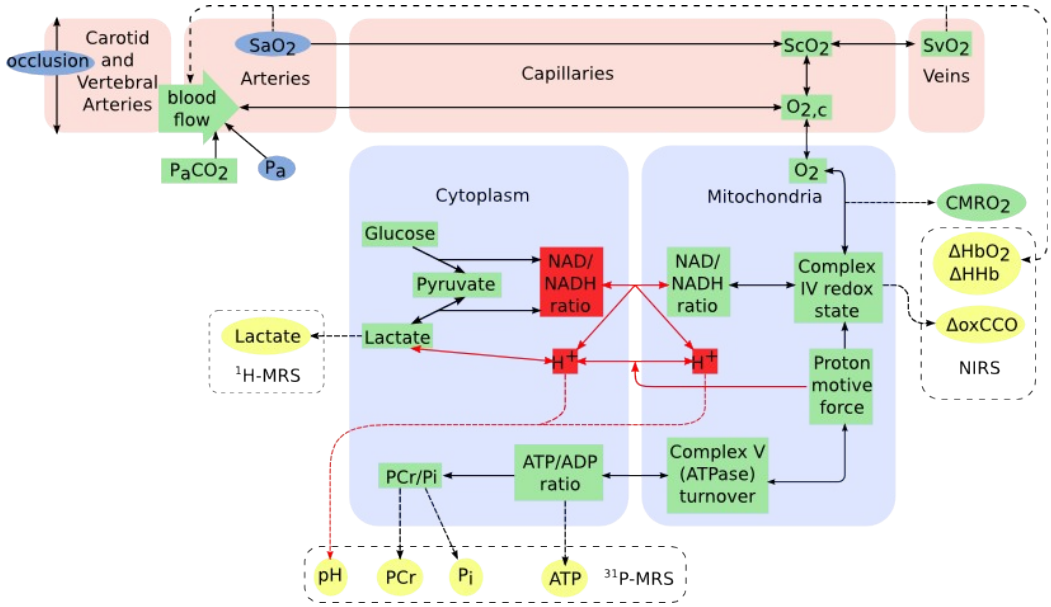

Figure 1: Schematic diagram of the model - inputs are shown in blue ovals, outputs in green and yellow ovals and model variables in rectangles. Modifications made in BrainPiglet v2 are shown in red.

2. Methods

2.1 Measurements

We monitor brain tissue physiology and biochemistry through a novel multimodal approach combining MRS and broadband NIRS. Broadband NIRS measures changes in cerebral oxygenation and volume. In particular, it detects oxygenated (HbO_2) and deoxgenated (HHb) haemoglobin concentrations, as well as the oxidation state of cytochrome-c-oxidase (ox-CCO). Cytochrome-c-oxidase (CCO) is the terminal electron acceptor of the mitochondrial electron transfer chain (ETC), which in turn provides a driving force for ATP synthesis. CCO therefore plays a crucial role in the dynamics of cellular energy production. As a complementary method, MRS (either proton (^{1}H) or phosphorus (^{31}P)) is used to observe variations in by-products of cellular metabolism, such as intracellular inorganic phosphate (Pi), phosphocreatine (PCr), adenosine triphosphate (ATP) and lactate (a marker of anaerobic metabolism). It can also detect

changes in intracellular pH and concentrations of N-acetylaspartate (NAA) - an abundant amino acid found mostly in neurons. In addition, as part of normal clinical practice, we continuously record systemic variables such as arterial blood pressure (Pa), arterial oxygen saturation (SaO_2), breathing rate and heart rate.

2.2 Experimental Protocol

All experiments were done under UK Home Office Guidelines (Animals [Scientific Procedures] Act, 1986) and were approved by the Institute of Neurology, University College London and UK Home Office. Here, we use recent data we have collected in piglets during HI. In this study baseline MRS and NIRS were acquired for 10mins; following this transient HI was induced by inflating vascular occluders that reduce flow in the carotid arteries, and initially reducing fractional inspired oxygen (FiO_2) to 12% for 4mins, then to 10% for 2mins, and then to 8% for 8mins (total 14mins). FiO_2 was then increased to 12% for 2mins, then 16% for 6mins and then 19% for 4.5mins (total 12.5mins) after which the occluders were deflated and FiO_2 normalised. During each study broadband NIRS and either ^{1}H MRS or ^{31}P MRS were acquired simultaneously every 1min during the baseline period, during HI and for a further 1h to monitor recovery from HI.

2.3 Modelling

Placing emphasis on the physiology of the brain, our model consists of a set of algebraic relations and differential equations describing cerebral blood flow and oxygenation, and oxygen and energy metabolism on a cellular level - including glycolysis and the tricarboxylic acid cycle. We have included different types of chemical reactions, such as mass action or, in the case of enzyme kinetics, Michaelis-Menten reactions. This system of equations incorporates 107 explicitly set parameters and 22 variables. Figure 1 illustrates a schematic diagram of the model. Arterial blood pressure (P_a), arterial oxygen saturation (SaO_2) and arterial carbon dioxide (P_aCO_2) are, where available, used as inputs. The model is then able to simulate changes in NIRS-measured quantities - HbO_2, HHb and oxCCO, and MRS-measured quantities - inorganic phosphate (Pi), phosphocreatine (PCr) and adenosine triphosphate (ATP). It also models changes in the cerebral metabolic rate of oxygen ($CMRO_2$) and lactate.

Blood flow is modelled as three compartments – arteries and arterioles, capillaries and veins – with varying conductances and radii. We have recently added an extra compartment to represent the supply of blood into the arteries. By varying the radius of this new compartment, we can simulate the carotid artery occlusion that results in ischaemia.

Brain pH is considered to be an important marker of neonatal health. Numerous experimental studies have shown a shift in intracellular brain pH following hypoxic-ischaemia, and attempts have been made to model pH in the recent past [6]. We have recently developed our model to simulate pH, by including the main dynamics of H+ ions in both cell mitochondria and cytoplasm. A number of reactions involving H+ ions have been modified, such as the TCA cycle, pyruvate-lactate conversion and the oxidative phosphorylation reactions. Cytoplasmic concentrations of NAD and NADH have been introduced. The malate-aspartate shuttle has also been included to facilitate the interchange of NAD/NADH and H+ ions between cell mitochondria and cytoplasm. These modifications are shown in Figure 1 in red.

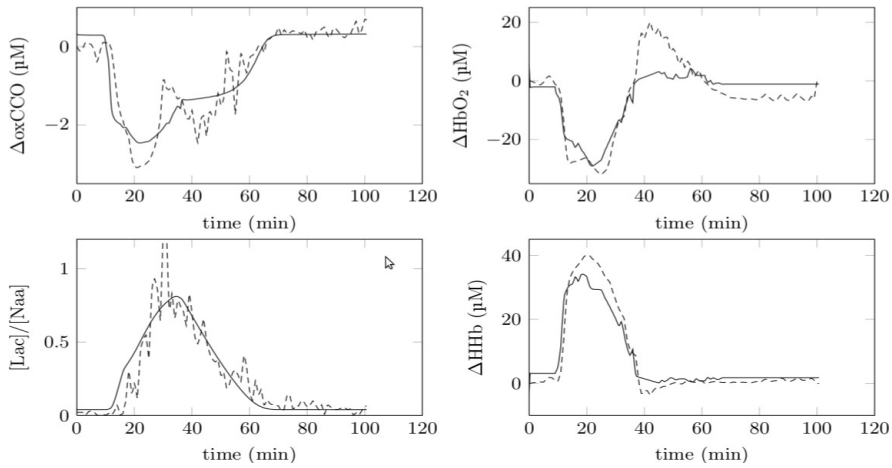

Figure 2: Model simulations (solid line) against NIRS and MRS measurements (dashed line) during hypoxia in piglets

3. Experimentation and Results

The model has been used to simulate measurements from experiments involving HI in newborn piglets, following the protocol detailed in section 2.2. P_a and SaO_2 were used as inputs to the model. Carbon dioxide (CO_2) concentration was not recorded in these experiments, however the piglets were ventilated with controlled levels of CO_2. Consequently, we have assumed that the partial pressure of CO_2 remains constant at 40 mmHg.

Results and modelled simulations from one HI experiment, using ^{1}H MRS are illustrated in Figure 2. While most model results fit well with the measurements observed, some did not – such as the increase in HbO_2 following the insult.

Figure 3 illustrates some of our results from another HI experiment. ^{31}P MRS was used here to measure pH changes in the brain. Both modelled HbO_2 and HHb (Figure3A and B) fit well with the NIRS measured data. Figure 3C illustrates modelled mitochondrial pH and cytoplasmic pH with MRS measured pH. We must note here that the quantity measured is an average pH value for the brain, and this appears to fit well with the modelled cytoplasmic pH. We expect

the relative volume of mitochondria in the brain to be quite small and so unlikely to have a significant effect on overall pH. In this case, by using parameter fitting techniques, our simulations were improved by increasing the parameter representing the total normal haemoglobin concentration in arteries and veins (from 5.4mM to 6.11mM)

We are currently investigating these similarities and differences between the modelled and measured variables to gain an insight into the biochemical processes that take place during an HI insult.

4. Discussion

The mathematical model of cerebral physiology and metabolism has been shown to successfully simulate oxygenation and metabolic changes during hypoxic-ischaemia episodes in piglets. It has the capacity to simulate broadband NIRS and MRS measured variables and infer some knowledge about the metabolic and circulatory processes that take place following a physiological insult.

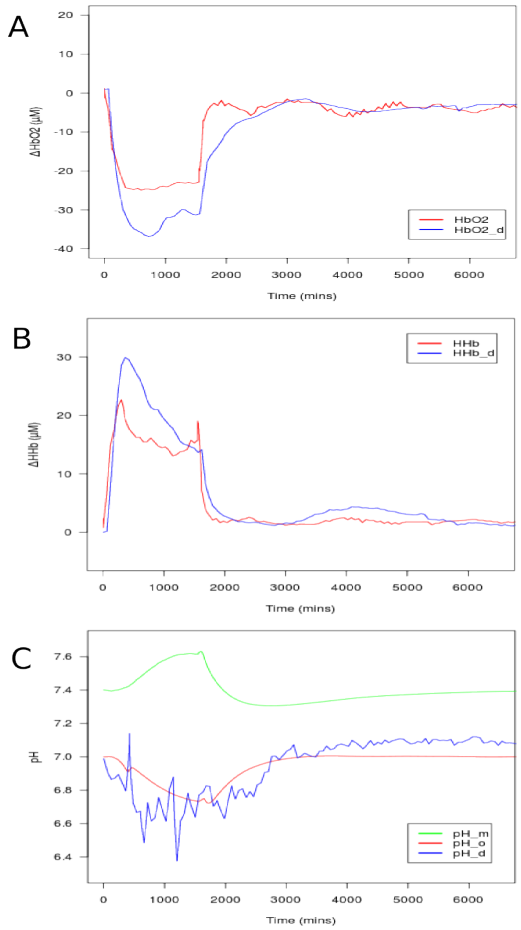

Figure 3: Model simulations (green and red) against NIRS and MRS measurements (blue) during hypoxia in piglets; In C, pH_m (green) represents modelled mitochondrial pH, pH_o (red) modelled cytoplasmic pH and pH_d (blue) MRS-measured brain pH.

Our recent developments – adapting the model to simulate pH and carotid artery occlusion – have produced good results. However, our experimental data is limited by the measurement techniques used. We are aware that the pH that is measured and that which is modelled are slightly different quantities; MRS provides an average measure of pH in an area of the brain including blood, tissue and various cells, while we model cytoplasmic and mitochondrial pH specifically. These differences must be acknowledged when comparing modelled and experimental results.

The BrainPiglet model is a useful interdisciplinary tool for attempting to understand the physiology and pathology of a complex organ. It is also a good method for validating our measurements and the techniques used to obtain them. Furthermore, our model has the capacity to test various neuroprotective strategies such as hypothermia, and other procedures that are employed clinically in the event of hypoxia.

As our model uses a physiological approach, expansions can increase the complexity of the model, making analysis more difficult. So far, we have relied on current biological knowledge and experimentation to set parameters and identify those with the most important effects. We are looking to apply rigorous sensitivity analysis methods to determine the effects of all parameters on the behaviour of the model. In future, we hope to investigate other methods of analysing our model and interpreting its results, and in doing so gain a better understanding of the physiological processes that take place during oxygen deprivation. In due course, the model will be adapted to the human neonatal brain.

References

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