Journal of Clinical Neuroscience 30 (2016) 31–38

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

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

Clinical Study

Blood mitochondrial enzymatic assay as a predictor of long-term outcome in severe traumatic brain injury

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ARTICLE INFO

Article history:
Received 13 August 2015
Accepted 25 October 2015

Keywords:
Blood mitochondrial enzymes
Complex I
Complex IV
Predictors of outcome
Pyruvate dehydrogenase complex
Severe traumatic brain injury

ABSTRACT

Recent studies have observed the central role of mitochondrial dysfunction in severe traumatic brain injury (sTBI). One hundred and seven sTBI patients (18–65 years old, presenting within 8 hours of injury) were randomised for a placebo controlled phase II trial of progesterone with or without hypothermia. We serially analysed blood mitochondrial enzymes (Complex I [C1], Complex IV [C4] and pyruvate dehydrogenase complex [PDH]) using a dipstick assay at admission and 7 days later for 37 patients, irrespective of assigned group. Favorable Glasgow Outcome Scale (GOS) at 1 year was associated with admission C1 levels above 0.19 μg, admission C4 levels above 0.19 μg and day 7 C1 levels above 0.17 μg, all per 25 μl of blood. Unfavorable GOS at 1 year was associated with admission serum PDH levels above 0.23 μg/25 μl of blood. Survivors at 1 year had significantly higher admission serum C1 levels above 0.19 μg/25 μl and day 7 C1 levels above 0.17 μg/25 μl. To our knowledge this is the first clinical trial associating blood mitochondrial enzymes with long-term outcome in sTBI. Serial monitoring and optimisation of blood C1, C4 and PDH levels could aid in prognostication and potentially guide in using mitochondrial targeted therapies. Blood mitochondrial enzymatic assay might suggest global reduction–oxidation status.

1. Introduction

Traumatic brain injury (TBI) induced neuropathology proceeds in a triphasic manner, consisting of a primary insult at the time of impact, repair mechanisms occurring in the minutes to days following and regenerative processes occurring in the months to years following injury [1]. The mitochondria play a central role in cerebral energy metabolism, intracellular calcium homeostasis, and reactive oxygen species generation and detoxification [2,3]. Mitochondrial dysfunction has been attributed to secondary mechanisms of neuronal cell death, and is mediated by uncoupling of oxidative phosphorylation and the electron transport chain (ETC) leading to the following: reduced adenosine triphosphate (ATP) synthesis; increased lactate production and resulting acidosis by increased metabolism of glucose via glycolysis (anaerobic respiration); cytochrome c and calcium induced apoptosis cascade; reduced metabolism of neurotransmitters such as glutamate leading to excitotoxicity; and increased reactive oxygen species mediated oxidative damage of vital macromolecules [2,4]. Brain mitochondrial respiratory function is affected within a few hours of injury and persists for 1–2 weeks [3,5–8]. Pyruvate dehydrogenase complex (PDH) is a rate-limiting enzyme of the mitochondrial tri-carboxylic-acid cycle (TCA) and is an important bridge between aerobic and anaerobic respiration [3,6]. Complex I (C1; nicotinamide adenine dinucleotide [NADH] dehydrogenase) is a rate-limiting enzyme for the ETC, which accepts and transfers electrons to coenzyme Q. Complex IV (C4; cytochrome oxidase) is a terminal step of the ETC where oxygen is the final receptor for electrons [9]. The mitochondrial ETC generates ATP by coupling electron transfer between an electron donor (NADH) and electron acceptor (oxygen), with the transfer of protons across the membrane to generate energy in form of ATP (Fig. 1) [9]. These mitochondrial enzymes are highly susceptible to oxidative damage [1,4,10].

Recent animal TBI models have described potential role of brain parenchymal PDH, C1 and C4 activity to be used as a surrogate marker of brain injury severity and long-term prognostication [1,10,9,6]. However, its role in clinical studies is limited owing to

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http://dx.doi.org/10.1016/j.jocn.2015.10.051
0967-5868/

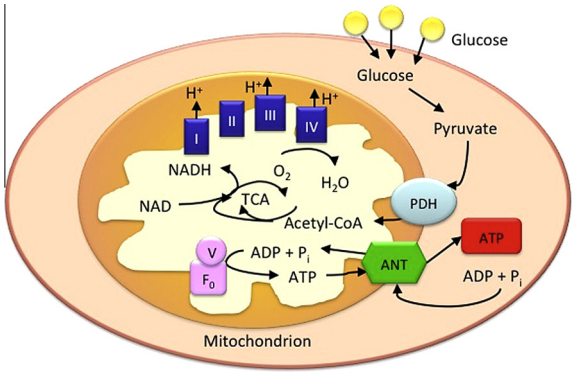

Fig. 1. Schematic diagram of the mitochondrial energy generating oxidative phosphorylation system. Acetyl CoA = acetyl coenzyme A, ADP = adenosine diphosphate, ANT = adenine nucleotide translocator, ATP = adenosine triphosphate, F0 = transmembrane proton channel for Complex V of electron transport chain, H + = proton ion, NAD = nicotinamide adenine dinucleotide (oxidized state), NADH = nicotinamide adenine dinucleotide (reduced state), PDH = pyruvate dehydrogenase complex, Pi = inorganic phosphate, TCA = tri-carboxylic acid cycle, I, II, III, IV and V represent enzymatic steps of electron transport chain.

the invasive nature of enzymatic assessment, probable sampling error related to its proximity to the traumatic lesion and physiological variation of enzymatic expression in different anatomical locations of the brain [1,6]. Contrary to that, blood enzymatic assessment is conceptually less invasive, more cost effective and reflects global oxidative stress [10], taking into account the important confounding factor of polytrauma, which is present in a high percentage of severe traumatic brain injury (sTBI) patients. To our knowledge, no clinical studies have assessed the association of these enzymatic parameters with overall long-term outcome. We serially analysed blood mitochondrial enzymes quantitatively, and assessed their long-term predictive value on functional outcome.

2. Methodology

2.1. Study design

This study was part of a prospective, randomised placebo controlled phase II trial of progesterone with or without hypothermia (factorial design, unpublished data). The study population included adult (aged 18–65 years) sTBI patients, presenting within 8 hours of injury.

Out of 107 sTBI patients randomised for this trial, we serially analysed three blood mitochondrial enzymes (PDH, C1 and C4) for 37 patients, irrespective of assigned group. The long-term predictive value of these blood biomarkers using a dichotomised Glasgow Outcome Scale (GOS) (poor recovery = GOS 1–3, good recovery = GOS 4–5), dichotomised Functional Independence Measure (FIM) (functionally dependent = FIM $\leqslant$108, functionally independent = FIM 109–126) and survival status (survivor, non-survivor) at 1, 6 and 12 month follow-up was analysed.

2.2. Dipstick test to determine total PDH enzyme, CI and C4 in blood samples

Blood C1, C4 and PDH were immunocaptured using a dipstick assay kit according to the manufacturer's guidelines (Abcam, Cambridge, UK) using an immunologic sandwich assay. The intensity of the colour band on the dipstick was measured using a flatbed scanner and the results were analyzed by J-image software. Quantitatively, the values are expressed as μg/25 μl of blood.

Sampling was done at admission and 1 week later. The dipstick method was performed rather than the conventional spectrophotometric method because of its faster response (within 30 minutes), no need for mitochondrial isolation, small size of the analytical kit, higher sensitivity to a smaller amount of target proteins and being more cost-effective [10].

2.3. Statistical analysis

Using the Statistical Package for the Social Science version 20 (IBM, Armonk, NY, USA), baseline parameters, blood mitochondrial enzymes and outcome were presented as number (percentage), mean (±standard deviation) or median (interquartile range), wherever appropriate. Comparative analysis for admission, day 7 and average serum biomarkers (stratified according to outcome) was performed using independent Student's t-test. Receiver-operating characteristic curve was created for factors predicting outcome along with estimation of area under the curve (AUC), sensitivity and specificity of optimal cut-off value.

3. Results

3.1. Demographic profile

A total of 37 patients (86.5% male) with a mean age of 30.8 years were analysed. Six, 12 and 15 patients were lost to follow-up at 1, 6 and 12 months, respectively. All the outcome analysis groups were similar with respect to the assigned treatment arm ($p > 0.05$), thereby reducing any potential bias arising from heterogeneous distribution of neuroprotective interventions (Table 1).

3.2. Comparative analysis of blood mitochondrial enzymes

At 12 months, the blood levels of C1 at admission, C4 at admission and C1 on day 7 were significantly higher in the GOS favorable outcome group (0.23 μg *versus* 0.14 μg, $p = 0.001$; 0.21 μg *versus* 0.17 μg, $p = 0.04$; and 0.18 μg *versus* 0.15 μg, $p = 0.02$, respectively). At 12 months, the blood levels of PDH at admission were significantly higher in the GOS unfavorable outcome group (0.25 μg *versus* 0.20 μg, $p = 0.001$) (Table 2).

The blood levels of C1, C4 and PDH were similar across both FIM groups at 12 months ($p > 0.05$) (Table 3).

At 12 months, the blood levels of C1 at admission and day 7 C1 were significantly higher in survivors than non-survivors (0.23 μg *versus* 0.14 μg, $p = 0.003$, and 0.18 μg *versus* 0.14 μg, $p = 0.02$, respectively) (Table 4).

3.3. Receiver operating characteristics curve analysis

Favorable GOS at 1 year was associated with admission C1 levels above 0.19 μg, admission C4 levels above 0.19 μg and day 7 C1 levels above 0.17 μg (Fig. 2). Unfavorable GOS at 1 year was associated with admission PDH levels above 0.23 μg.

Receiver operating characteristics curve for FIM admission and day 7 are shown in Figure 3.

Survivors at 1 year had significantly higher admission C1 levels above 0.19 μg and day 7 C1 levels above 0.17 μg (Fig. 4, Table 5).

4. Discussion

4.1. Blood enzymatic assay for mitochondrial enzymes

The blood enzymatic assay primarily assesses the activity of enzymes in viable mitochondria, which are present in mononuclear cells found in peripheral blood [10]. We believe that global