Experimental Neurology 261 (2014) 109–119

Contents lists available at ScienceDirect

Experimental Neurology

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

Regular Article

Associations between in vivo neuroimaging and postmortem brain cytokine markers in a rodent model of Wernicke's encephalopathy

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ARTICLE INFO

Article history:
Received 9 April 2014
Revised 13 June 2014
Accepted 17 June 2014
Available online 25 June 2014

Keywords:
Thiamine
TDP
Lactate
Microglia
MCP-1
Magnetic resonance imaging
Magnetic resonance spectroscopy
Immunohistochemistry
Liver
Brain

ABSTRACT

Thiamine (vitamin B1) deficiency, associated with a variety of conditions, including chronic alcoholism and bariatric surgery for morbid obesity, can result in the neurological disorder Wernicke's encephalopathy (WE). Recent work building upon early observations in animal models of thiamine deficiency has demonstrated an inflammatory component to the neuropathology observed in thiamine deficiency. The present, multilevel study including in vivo magnetic resonance imaging (MRI) and spectroscopy (MRS) and postmortem quantification of chemokine and cytokine proteins sought to determine whether a combination of these in vivo neuroimaging tools could be used to characterize an in vivo MR signature for neuroinflammation. Thiamine deficiency for 12 days was used to model neuroinflammation; glucose loading in thiamine deficiency was used to accelerate neurodegeneration. Among 38 animals with regional brain tissue assayed postmortem for cytokine/chemokine protein levels, three groups of rats (controls + glucose, n = 6; pyrithiamine + saline, n = 5; pyrithiamine + glucose, n = 13) underwent MRI/MRS at baseline (time 1), after 12 days of treatment (time 2), and 3 h after challenge (glucose or saline, time 3). In the thalamus of glucose-challenged, thiamine deficient animals, correlations between in vivo measures of pathology (lower levels of N-acetyle aspartate and higher levels of lactate) and postmortem levels of monocyte chemotactic protein-1 (MCP-1, also known as chemokine ligand 2, CCL2) support a role for this chemokine in thiamine deficiency-related neurodegeneration, but do not provide a unique in vivo signature for neuroinflammation.

Introduction

Mounting evidence, primarily from experimental models of conditions including Alzheimer's disease (e.g., McLarnon, 2014), Parkinson's disease (e.g., Sanchez-Guajardo et al., 2013), schizophrenia (e.g., Upthegrove et al., 2014), and major depressive disorder (e.g., Najjar et al., 2014), suggests that inflammatory processes play a role in central nervous system (CNS) pathology. Indeed, it has been suggested that molecules released by injured neurons can interact with activated microglia to trigger a self-perpetuating cycle of injury with prolonged microglial activation that contributes to neurodegeneration (Block and Hong, 2005). Identifying in vivo, non-invasive markers capable of dissociating neuroinflammatory and neurodegenerative processes would be valuable for longitudinally evaluating progression of diseases with inflammatory components and the effects of therapeutic interventions. Magnetic resonance imaging (MRI) and spectroscopy (MRS) can assess the structure and chemical composition of the brain in vivo and together have the potential to be used as biomarkers for neuroinflammation.

The acute neuropsychiatric consequence of thiamine (vitamin B1) deficiency, Wernicke's encephalopathy (WE), is associated with conditions such as alcoholism, bariatric surgery for morbid obesity (Berger and Singhal, 2014; Merola et al., 2012), cancer, HIV/AIDS, and advanced age (Lee et al., 2000). Animal models support a contribution of classical sterile neuroinflammatory mechanisms (cf., Graeber et al., 2011) in thiamine deficiency related pathology. Indeed, neuropathologic consequences of thiamine deficiency include blood–brain barrier breakdown (Beauchesne et al., 2009; Calingasan and Gibson, 2000; Ke and Gibson, 2004; Nixon et al., 2008) and accumulation of innate and adaptive immune cells in CNS tissue (McRee et al., 2000; Meng and Okeda, 2003). Alterations in glial cell morphology were reported in early studies of experimental thiamine deficiency (Collins, 1967;

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http://dx.doi.org/10.1016/j.expneurol.2014.06.015
0014-4886/

Robertson et al., 1968; Tellez and Terry, 1968) and in human WE (Victor et al., 1971).Activated macrophages/microglia have been noted in several vulnerable brain regions (Todd and Butterworth, 1999). Further, in the vulnerable thalamus (Karuppagounder et al., 2007; Vemuganti et al., 2006) and inferior colliculus (Vemuganti et al., 2006), cytokine genes and proteins (e.g., IL-1β, TNF-α) have been shown to be upregulated. Although inflammation reflecting neuronal damage may appear late in the temporal sequence of events following thiamine deficiency, cytokine signaling may spread damage to vulnerable brain regions (Ke and Gibson, 2004; Ke et al., 2005). It has been proposed that the sequence of events leading to neurodegeneration in thiamine deficiency begins with oxidative stress causing neuronal secretion of monocyte chemoattractant protein-1 (MCP-1) that activates microglia to secrete cytokines that then promote neuronal death (Yang et al., 2011).

Studies have attempted to demonstrate relationships between in vivo MR metrics and postmortem markers of neuroinflammation. For example, volumes of T2-weighted hyperintensities in an animal model of multiple sclerosis were shown to correlate with inflammatory cell accumulation (Tourdias et al., 2011). In a model of hydrocephalus, ventricular enlargement was associated with increased expression of GFAP (a marker of astrocytosis) and Iba-1 (a marker of microgliosis) (Xu et al., 2012). MRS in neuroinflammatory conditions such as multiple sclerosis and HIV typically identifies a pattern of reduced levels of N-acetyl aspartate (NAA, a marker of neuronal integrity) (Rigotti et al., 2012; Schweinsburg et al., 2005), elevated levels of choline-containing compounds (Cho, a marker of cell-membrane metabolism and cellular turnover) (Inglese et al., 2003; Mader et al., 2008; Valcour et al., 2012), and elevated levels of myo-Inositol (mI, a putative glial marker) (Bagory et al., 2012; Fernando et al., 2004; Harezlak et al., 2011; Kirov et al., 2009).

By using both in vivo MRI and MRS and postmortem quantification of chemokine/cytokine protein levels in several brain regions, the current study sought to determine relationships between in vivo MR markers of pathology and molecules associated with inflammation with the intention of determining an in vivo MR signature for neuroinflammation. Three groups of animals were included: control animals, thiamine-deficient animals challenged with saline (to model neuroinflammation in the absence of severe neurodegeneration), and thiamine-deficient animals challenged with glucose (to model neurodegeneration) (Jordan et al., 1998). Measurement of whole blood levels of thiamine and its phosphate derivatives was used to index severity of thiamine deficiency.

Methods

Animals

The Institutional Animal Care and Use Committees (IACUC) at SRI International and Stanford University approved all research protocols in accordance with NIH IACUC guidelines, and the Guide for the Care and Use of Laboratory Animals (Committee, 2010). A total of 56 adult, male, wild-type Wistar rats weighing 280.5 ± 4.8 g at study entry were individually housed with free access to food and water. Animals used in these experiments were maintained in fully accredited facilities. The number of animals used in specific assays is presented in Table 1.

Table 1
Number of animals in each assay.

	Thiamines	MR imaging	Liver histology	Brain histology	Brain cytokines
Thiamines	56	24	56	10	38
MR imaging	24	24	24	8	22
Liver histology	56	24	56	10	38
Brain histology	10	8	10	10	10
Brain cytokines	38	22	38	10	38

Thiamine deprivation treatment

Following the protocol employed in prior studies (Ciccia and Langlais, 2000; Langlais and Zhang, 1997; Pfefferbaum et al., 2007; Pitkin and Savage, 2001), all 56 rats received thiamine-deficient chow (Harlan-Teklad, Madison, WI, TD81029). Control animals (n = 19) additionally received daily intraperitoneal (IP) injections of thiamine hydrochloride (0.4 mg/kg). The remaining 37 animals received daily IP injections of pyrithiamine hydrobromide (0.5 mg/kg): pyrithiamine-treated (PT) rats were made thiamine-deficient until ataxia was observed, typically by day 12 of treatment. A glucose challenge was given to accelerate neurodegeneration (Harper, 1980; Jordan et al., 1998; Victor et al., 1989; Wallis et al., 1978; Watson et al., 1981). All 19 thiamine treated (i.e., control) animals were challenged with glucose (5 g/kg) by intravenous (IV) administration in the caudal tail vein (i.e., control + glucose, CG), 10 pyrithiamine-treated animals received IV saline (i.e., pyrithiamine + saline, PS), and 27 pyrithiamine-treated animals were challenged with IV glucose (i.e., pyrithiamine + glucose, PG). All chemicals were obtained from Sigma Aldrich, Saint Louis, MO.

Behavioral analysis

Neurological examination (Becker, 2000; Roberts et al., 1996; Yaksh et al., 1977) was performed daily on 19 CG and 37 PT animals beginning on day 8 and continuing daily until day 12 or 13 of thiamine deprivation. Rats were rated (0 = absent, 1 = present) for the presence of behavioral signs in the following categories: autonomic, sensory, arousal, posture, motor, central, and responses to stimulation.

Measurement of thiamine and its derivatives

Whole blood (~1.5 ml) was collected via retro-orbital bleed in EDTA tubes at baseline (11 CG, 23 PT) and after 12–13 days of thiamine deficiency (19 CG, 37 PT). Of the 27 animals that received glucose challenge, 10 had blood collected after the challenge to determine if glucose challenge further affected levels of thiamine and its derivatives. AniLyitics Inc. (Gaithersburg, MD), measured thiamine and its phosphate derivatives (thiamine monophosphate (TMP) and thiamine diphosphate (TDP)) using recommended methods (Mancinelli et al., 2003).

MR scanning procedures and data analysis

Schedule

MRI and MRS were acquired on 24 animals (6 CG, 5 PS, 13 PG) at baseline (time 1), on days 12 or 13 of thiamine deprivation (time 2), and within 3 h of saline or glucose challenge (time 3), administered immediately upon completion of the 2nd scan (Fig. 1a). All 56 animals, whether they were MR scanned or not, were sacrificed within 6 h of the challenge.

Anesthesia and monitoring

Animals were held in an MR-invisible structure providing support for a radiofrequency (RF) coil and nose cone for delivery of isoflurane anesthesia (1.5–3%) and oxygen (1.5 L/min) (Adalsteinsson et al., 2004). Blood oxygen saturation, pulse rate, rectal temperature, and respiration were monitored throughout the ~2 h MR scan.

MRI acquisition

Imaging was conducted on a clinical 3T GE Signa MR scanner equipped with a high-strength insert gradient coil (peak strength = 600 mT/m, peak slew rate = 3200 T/m/s; (Chronik et al., 2000a, b) operated at 500 mT/m and a slew rate of 1800 mT/m/ms). A custom-made rat brain quadrature head coil (Ø = 44 mm) was used for both RF excitation and signal reception. A gradient-recalled echo localizer scan was used to position the animals in the scanner and for graphical prescription of the subsequent scans. High resolution, dual-echo, fast