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Research Report

24S-hydroxycholesterol effects on lipid metabolism genes are modeled in traumatic brain injury

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ABSTRACT

Membrane damage during traumatic brain injury (TBI) alters the brain homeostasis of cholesterol and other lipids. Cholesterol 24S-hydroxylase (Cyp46) is a cholesterol metabolic enzyme that is increased after TBI. Here, we systematically examined the effects of the enzymatic product of Cyp46, 24S-hydroxycholesterol, on the cholesterol regulatory genes, SREBP-1 and 2, their posttranslational regulation, and their effects on gene transcription. 24S-hydroxycholesterol increased levels of SREBP-1 mRNA and full-length protein but did not change levels of cleaved SREBP-1, consistent with the role of 24-hydroxycholesterol as an LXR agonist. In contrast, 24S-hydroxycholesterol decreased levels of LXR-independent SREBP-2 mRNA, full-length protein, and SREBP-2 active cleavage product. We examined the downstream effects of changes to these lipid regulatory factors by studying cholesterol and fatty acid synthesis genes. In neuroblastoma cells, 24S-hydroxycholesterol decreased mRNA levels of the cholesterol synthesis genes HMG CoA reductase, squalene synthase, and FPP synthase but did not alter levels of the mRNA of fatty acid synthesis genes acetyl CoA carboxylase or fatty acid synthase. After TBI, as after 24S-hydroxycholesterol treatment in vitro, SREBP-1 mRNA levels were increased while SREBP-2 mRNA levels were decreased. Also similar to the in vitro results with 24S-hydroxycholesterol, HMG CoA reductase and squalene synthase mRNA levels were significantly decreased. Fatty acid synthase mRNA levels were not altered but acetyl CoA carboxylase mRNA levels were significantly decreased. Thus, changes to transcription of cholesterol synthesis genes after TBI were consistent with increases in Cyp46 activity, but changes to fatty acid synthesis genes must be regulated by other mechanisms.

1. Introduction

Cholesterol 24S-hydroxylase (Cyp46) is a brain-enriched enzyme expressed primarily in neurons; it is responsible for converting cholesterol from the plasma membrane into 24S-hydroxycholesterol (Lund et al., 1999; Ramirez et al., 2008). Expression of Cyp46 in the brain is altered during normal development but is relatively stable in normal adult brain (Lund et al., 1999; Ohyama et al., 2006). Cyp46 is increased after traumatic brain injury (TBI), specifically in microglia (Cartagena et al., 2008). Other models of brain injury including hippocampal kainate injury (He et al., 2006) and acute experimental

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autoimmune encephalomyelitis (Teunissen et al., 2007) have shown increases in Cyp46 expression at lesion sites. The enzymatic product of Cyp46, 24S-hydroxycholesterol, is increased in the CSF of Alzheimer's disease (AD) patients compared to control subjects (Papassotiropoulos et al., 2002). These findings suggest that while Cyp46 activity in neurons plays a critical role in normal cholesterol homeostasis, increased Cyp46 activity may be a characteristic of the brain response to injury.

Cholesterol synthesized outside the CNS cannot enter the brain across the blood–brain barrier (Jurevics and Morell, 1995; Turley et al., 1996). The brain synthesizes cholesterol and cholesterol turnover rates indicate that cholesterol exits the CNS at a rate of 1.4 mg/day/kg in the mouse (Dietschy and Turley, 2004). Cyp46 knockout mouse studies indicate that the conversion of cholesterol to 24S-hydroxycholesterol accounts for 64% of cholesterol efflux out of the brain (Dietschy and Turley, 2004; Xie et al., 2003). The remainder of cholesterol efflux out of the brain may be explained by inclusion of lipoproteins containing cholesterol and apolipoprotein E (apoE) (Pitas et al., 1987) in bulk CSF outward flow through the arachnoidal–lymphatic–venous interfaces (Johanson et al., 2008). 24S-hydroxycholesterol is also an activator of the nuclear transcription factors liver X receptors (LXR) α and β (Janowski et al., 1999; Lehmann et al., 1997) and can induce the upregulation of genes involved in cellular cholesterol efflux (Rebeck, 2004; Tall, 2008). LXR activation increases expression of genes important for cholesterol efflux such as ATP-binding cassette transporter (ABC) A1 in both neurons and glia (Fukumoto et al., 2002) and apoE in astrocytes (Liang et al., 2004). We have shown increases in both apoE and ABCA1 coinciding with increased Cyp46 expression after TBI (Cartagena et al., 2008). When both apoE and ABCA1 are present, cholesterol can be passed from the cell membrane to extracellular lipoproteins (Hirsch-Reinshagen et al., 2005; Huang et al., 2001). These cholesterol efflux mechanisms are important for lowering cholesterol levels when cellular cholesterol is in excess. In addition, the conversion of cholesterol to 24S-hydroxycholesterol makes it more soluble and easier to clear into the extracellular space and across the blood–brain barrier into the blood (Bjorkhem, 2006; Olkkonen and Hynynen, 2009; Vaya and Schipper, 2007). Thus, 24S-hydroxycholesterol allows for two separate mechanisms for clearing cholesterol from the cell and from the brain overall.

24S-hydroxycholesterol potentially plays a role in regulating the cholesterol and fatty acid synthesis pathways in the brain both by its LXR activity and also because it is an oxysterol. Oxysterols alter lipid synthesis mechanisms by acting on sterol regulatory element (SRE) binding proteins (SREBPs). SREBPs are expressed as inactive 120 kDa precursors (pSREBP) which are integral to the endoplasmic reticulum (ER) membrane. pSREBPs are translocated from the ER to the Golgi by SREBP cleavage-activating protein (SCAP) where they are cleaved into a 67-kDa active transcription factor, which is not membrane bound. This shorter mature SREBP (mSREBP) alters transcription of genes containing an SRE in the promoter region. These genes are responsible for critical enzymes in both the cholesterol synthesis pathway and the fatty acid synthesis pathway. When intracellular cholesterol levels are in excess, SCAP, which has a cholesterol sensing domain, binds insulin induced gene (Insig) and the Insig–SCAP–pSREBP is retained in the ER (Olkkonen and Hynynen, 2009; Radhakrishnan et al., 2007). Oxysterols synthesized outside the CNS, such as 25-hydroxycholesterol, have been shown to suppress the cleavage of pSREBP to mSREBP (Adams et al., 2004; Du et al., 2004; Thewke et al., 1998) and to bind Insig rather than SCAP (Sun et al., 2007) and induce Insig–SCAP binding (Adams et al., 2004), thus retaining SCAP in the ER and preventing pSREBP translocation to the Golgi for cleavage (Olkkonen and Hynynen, 2009; Radhakrishnan et al., 2007).

There are two genes for SREBPs giving rise to the three isoforms: 1a, 1c, and 2. SREBP-1c differs from SREBP-1a only in a small portion of exon 1 but they are otherwise identical (Brown and Goldstein, 1997). SREBP-1a is constitutively expressed (Raghow et al., 2008). SREBP-2 has been shown to contain an SRE in its promoter region which, when bound, increased transcription of SREBP-2 (Sato et al., 1996). Neither SREBP1a nor SREBP-2 is known to be regulated by LXR. In contrast, SREBP-1c has been shown to be under LXR control (Whitney et al., 2002) and also contains a SRE in its promoter region (Cagen et al., 2005). In both liver and brain, expression levels of SREBP-1c are greater than SREBP-1a (Shimomura et al., 1997). In mice expressing transgenes for mature forms of SREBP-1a, SREBP-1c, and SREBP-2, mSREBP-1c (expressed primarily in liver) has weak transcription activity in comparison to the other isoforms on downstream cholesterol and fatty acid synthesis gene expression, while mSREBP-1a preferentially increases expression of fatty acid synthesis genes and mSREBP-2 preferentially increases expression of cholesterol synthesis genes (Horton and Shimomura, 1999; Horton et al., 1998).

Various oxysterols differ in their biological effects (Gill et al., 2008). For example, 27-hydroxycholesterol is an LXR agonist (Fu et al., 2001), while 25-hydroxycholesterol has only minimal LXR activity (Janowski et al., 1999). In the CYP27 –/– mouse liver and adrenals, SREBP-2 mRNA levels were increased as were mRNA levels of HMG CoA synthase and HMG CoA reductase and cholesterol synthesis (Repa et al., 2000a,b,c). This supports the hypothesis that products of CYP27, including 27-hydroxycholestrol, have inhibitory effects on cholesterol synthesis (Lund et al., 1993). 24S-hydroxycholesterol is the main oxysterol in the brain (Dietschy and Turley, 2004; Karu et al., 2007). In the CYP46 –/– mouse, cholesterol excretion from the brain is reduced, as is cholesterol synthesis, leading to stable total brain cholesterol levels (Xie et al., 2003). These data conflict with the hypothesis that 24S-hydroxycholesterol inhibits the cholesterol synthesis pathway through effects on SREBP cleavage. It is possible that altering one mechanism of cholesterol metabolism leads to compensation in other pathways. For example, CYP27 has been shown to synthesize several oxysterols including 24-hydroxycholesterol (Lund et al., 1993) and transgenic mice overexpressing CYP27 have decreased circulating levels of 24S-hydroxycholesterol (Meir et al., 2002).

Microglia express CYP27 (Gilardi et al., 2009) and express CYP46 after TBI (Cartagena et al., 2008). Astrocytes increase CYP27 expression with LXR agonist treatment (Gilardi et al., 2009) and express CYP46 in AD brain (Bogdanovic et al., 2001; Brown et al., 2004). In astrocytes, 24S-hydroxycholesterol decreased expression of the rate-limiting step in cholesterol synthesis, HMG CoA reductase, and increased LXR-regulated apoE expression (Abildayeva et al., 2006). In cortical neurons, 24S-hydroxycholesterol decreased expression of cholesterol