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Research Report

White matter tract and glial-associated changes in 5-hydroxymethylcytosine following chronic cerebral hypoperfusion

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ABSTRACT

White matter abnormalities due to age-related cerebrovascular alterations is a common pathological hallmark associated with functional impairment in the elderly which has been modeled in chronically hypoperfused mice. 5-Methylcytosine (5mC) and its oxidized derivative 5-hydroxymethylcytosine (5hmC) are DNA modifications that have been recently linked with age-related neurodegeneration and cerebrovascular pathology. Here we conducted a pilot investigation of whether chronic cerebral hypoperfusion might affect genomic distribution of these modifications and/ or a Ten-Eleven Translocation protein 2 (TET2) which catalyses hydroxymethylation in white and grey matter regions of this animal model. Immunohistochemical evaluation of sham and chronically hypoperfused mice a month after surgery revealed significant ($p<0.05$) increases in the proportion of 5hmC positive cells, Iba1 positive inflammatory microglia, and NG2 positive oligodendroglial progenitors in the hypoperfused corpus callosum. In the same white matter tract

Abbreviations: CC1, adenomatous polyposis coli; CNS, central nervous system; DIV, days in vitro; DNMT1, DNA methyltransferase 1; GFAP, glial fibrillary acidic protein; HCl, hydrochloric acid; HDAC1, histone deacetylase 1; H&E, haemotoxylin and eosin; 5hmC, 5-hydroxymethylcytosine; Iba1, ionized calcium binding antigen 1; IFNγ, interferon γ; LPS, lipopolysaccaride; 5mC, 5-methylcytosine; NG2, chondroitin sulfate proteoglycan; OPCs, oligodendroglial progenitor cells; PBS, phosphate buffer saline; PLP, proteolipid protein; ssDNA, single stranded DNA; TET1, ten–eleven translocation protein 1; TET2, ten–eleven translocation protein 2; TET3, ten–eleven translocation protein 3

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there was an absence of hypoperfusion-induced alterations in the proportion of 5mC, TET2 positive cells and CC1 positive mature oligodrendrocytes. Correlation analysis across animals within both treatment groups demonstrated a significant association of the elevated 5hmC levels with increases in the proportion of inflammatory microglia only ($p=0.01$) in the corpus callosum. In vitro studies revealed that 5hmC is lost during oligodendroglial maturation but not microglial activation. Additionally, TET1, TET2, and TET3 protein levels showed dynamic alterations during oligodendroglial development and following oxidative stress in vitro. Our study suggests that 5hmC exhibits white matter tract and cell type specific dynamics following chronic cerebral hypoperfusion in mice.

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1. Introduction

White matter abnormalities are a common pathological feature of the ageing brain and are closely linked with cognitive impairment particularly a reduction in the speed of information processing (O'Sullivan et al., 2001; Charlton et al., 2006; Grieve et al., 2007; Kennedy and Raz, 2009; Bolandzadeh et al., 2012). Chronic cerebral hypoperfusion is closely associated with white matter alterations in ageing and in disease (Kalaria, 1996; Tang et al., 1997; de Leeuw et al., 2000; Farkas and Luiten, 2001; Fernando et al., 2006; Holland et al., 2008; DeCarli, 2013). To experimentally study the association between reductions in the cerebral blood supply and white matter pathology, animal models of chronic cerebral hypoperfusion have been developed initially in the rat and recently in the mouse. In the rat, chronic cerebral hypoperfusion is induced by the permanent bilateral ligation of the common carotid leading to 30–60% of baseline level initial reductions of the cerebral blood supply with a gradual flow recovery by 4 weeks post-surgery and the development of both white and grey matter pathology (Pappas et al., 1996; Abraham and Lazar, 2000; Wakita et al., 2002; Farkas et al., 2004, 2007; Otori et al., 2003; Tomimoto et al., 2003). Because of the occurrence of mixed white and grey matter abnormalities, it is difficult to examine the selective effects of chronic cerebral hypoperfusion on white matter integrity in chronically hypoperfused rats. Recently, ours and other groups developed a new mouse model of chronic cerebral hypoperfusion where wire microcoils with internal diameter of 0.18 mm are implanted around the common carotid arteries leading to mild (~20–30% of baseline levels) reductions of the cerebral blood supply and the development of a selective white matter pathology one month post-surgery (Shibata et al., 2004, 2007; Coltman et al., 2011; Holland et al., 2011; Reimer et al., 2011; McQueen et al., 2014). The observed pathological differences between chronically hypoperfused rats and mice could be explained by (1) the above mentioned methodological differences in the induction of chronic cerebral hypoperfusion as well as by (2) species differences in the cerebrovasculature (e.g. the Willy's polygon). In the rat, the Willy's Polygon is morphologically similar to this cerebrovascular structure in humans, whereas in the mouse the Willy's Polygon is significantly underdeveloped. The reported progressive recovery of the cerebral blood flow in chronically hypoperfused rats is explained by compensatory mechanisms such as enlargement of the posterior vessels constituting the Willy's Polygon (the posterior cerebral artery, the posterior communicating artery, and the basilar artery) between 3 and 6 months after the induction of chronic cerebral hypoperfusion (Olendorf, 1989; Choy et al., 2006).

Since selective white matter pathology in the absence of grey matter abnormalities develops in chronically hypoperfused mice (Shibata et al., 2004, 2007; Coltman et al., 2011; Holland et al., 2011; Reimer et al., 2011; McQueen et al., 2014), we used this animal model to examine molecular alterations occurring in the hypoperfused white matter. Specifically, molecular analysis of the underlying gene alterations evoked by hypoperfusion in the white matter demonstrated significant changes in the expression of 129 genes and highlighted aberrations in pathways linked to oxidative stress and inflammation (Reimer et al., 2011). However, the exact molecular mechanisms leading to transcriptional alterations in the hypoperfused white matter remain unclear. One possible explanation may rely on hypoperfusion-induced epigenetic changes as it is known that during normal development as well as under different pathological conditions, epigenetic marks can switch genes on and off (Murrell et al., 2013; MacDonald and Roskams, 2009).

5-Methylcytosine (5mC) is an epigenetic modification generated by addition of a methyl group to the 5′ carbon of cytosine that occurs predominantly in a CpG dinucleotide context (Bird, 2002). DNA methylation is mainly associated with transcriptional repression modulating cellular function during lifespan and disease. With ageing, dynamic global and gene-specific changes in 5mC distribution are observed in grey and white matter (West et al., 1995; Mehler, 2008; Zawia et al., 2009; Chouliaras et al., 2010; Penner et al., 2010; Hernandez et al., 2011; Chouliaras et al., 2012b; Coppieters et al., 2014). Several mechanisms may impact on DNA methylation with increasing age including cerebrovascular conditions (e.g. stroke, chronic cerebral hypoperfusion) and the resulting hypoxic-ischemic environment accompanied by increased excitotoxicity, oxidative stress, and inflammation (Endres et al., 2000; Westberry et al., 2008). Severe reductions of cerebral blood flow occurring as a result of experimental stroke and focal ischemia are known to reduce the levels of DNA methylation in the brain (Endres et al., 2000; Westberry et al., 2008). The exact functional role of DNA methylation in the injured central nervous system (CNS) is still controversial. For instance, although pharmacological and genetic inhibition