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Research paper

2-Chlorohexadecanoic acid induces ER stress and mitochondrial dysfunction in brain microvascular endothelial cells

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ABSTRACT

Peripheral leukocytes induce blood-brain barrier (BBB) dysfunction through the release of cytotoxic mediators. These include hypochlorous acid (HOCl) that is formed via the myeloperoxidase-H_2O_2-chloride system of activated phagocytes. HOCl targets the endogenous pool of ether phospholipids (plasmalogens) generating chlorinated inflammatory mediators like e.g. 2-chlorohexadecanal and its conversion product 2-chlorohexadecanoic acid (2-ClHA). In the cerebrovasculature these compounds inflict damage to brain microvascular endothelial cells (BMVEC) that form the morphological basis of the BBB. To follow subcellular trafficking of 2-ClHA we synthesized a 'clickable' alkyne derivative (2-ClHyA) that phenocopied the biological activity of the parent compound. Confocal and superresolution structured illumination microscopy revealed accumulation of 2-ClHyA in the endoplasmic reticulum (ER) and mitochondria of human BMVEC (hCMEC/D3 cell line). 2-ClHA and its alkyne analogue interfered with protein palmitoylation, induced ER-stress markers, reduced the ER ATP content, and activated transcription and secretion of interleukin (IL)−6 as well as IL-8. 2-ClHA disrupted the mitochondrial membrane potential and induced procaspase-3 and PARP cleavage. The protein kinase R-like ER kinase (PERK) inhibitor GSK2606414 suppressed 2-ClHA-mediated activating transcription factor 4 synthesis and IL-6/8 secretion, but showed no effect on endothelial barrier dysfunction and cleavage of procaspase-3. Our data indicate that 2-ClHA induces potent lipotoxic responses in brain endothelial cells and could have implications in inflammation-induced BBB dysfunction.

1. Introduction

The neurovascular unit separates most regions of the brain from the peripheral circulation to maintain the specialized central nervous system (CNS) micromilieu [1]. Brain microvascular endothelial cells (BMVEC) form the morphological basis of the blood-brain barrier (BBB) by the formation of tight junction (TJ) and adherens junction complexes [2]. These junctional complexes inhibit paracellular leakage and maintain CNS homeostasis via polarized expression of transporter systems taking a central biochemical gate-keeping function at the BBB [3,4].

Under inflammatory conditions BBB function is compromised and

Abbreviations: HA, hexadecan-1-oic (palmitic) acid; HyA, hexadec-15-yn-1-oic acid; 2-ClHDA, 2-chlorohexadecan-1-al; 2-ClHDyA, 2-chlorohexadec-15-yn-1-al; 2-ClHA, 2-chlorohexadecan-1-oic acid; 2-ClHyA, 2-chlorohexadec-15-yn-1-oic acid

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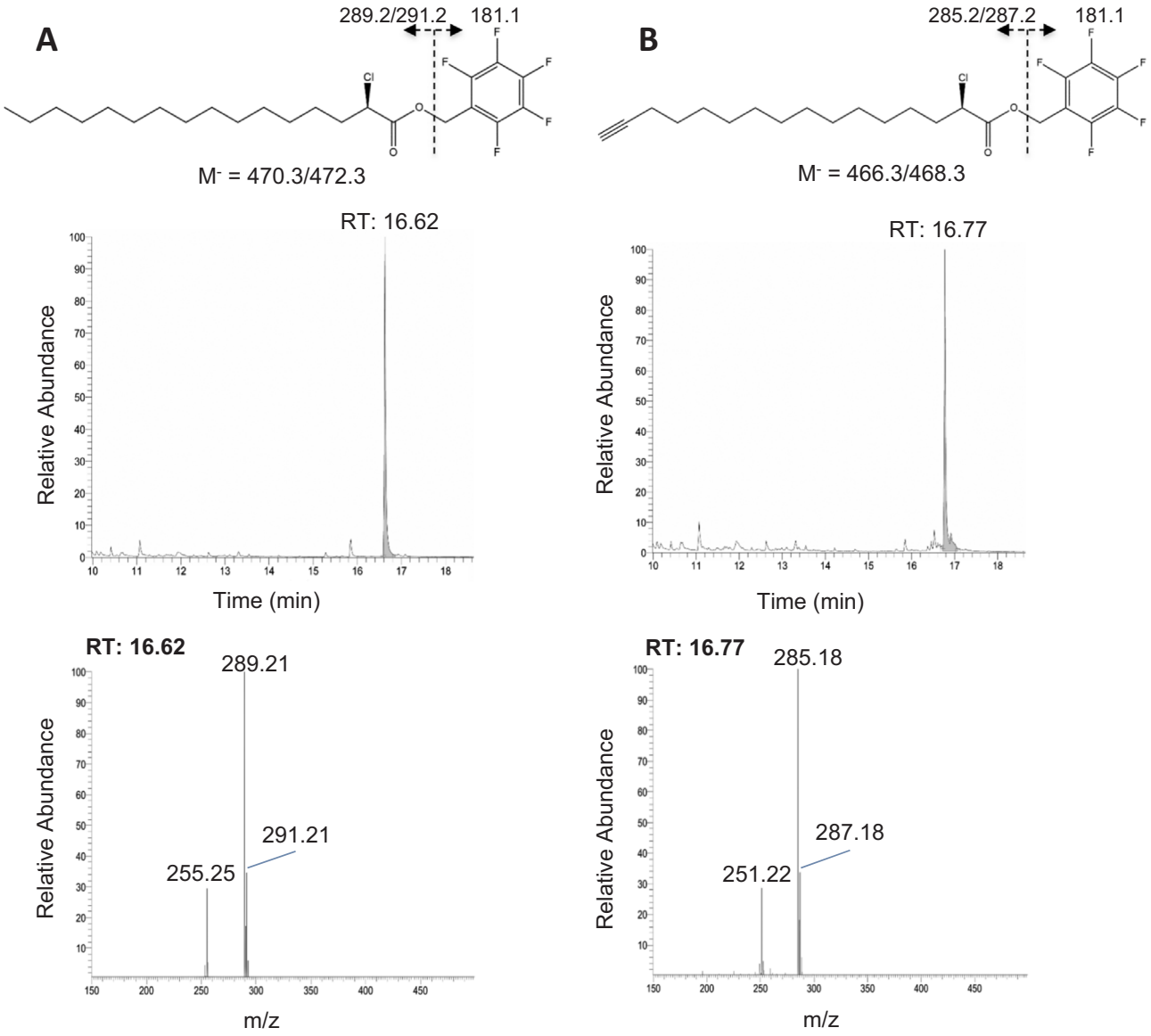

Fig. 1. NICI-GC-MS characterization of the PFB-ester derivatives of 2-ClHA and 2-ClHyA. 2-ClHA and 2-ClHyA were converted to the corresponding PFB ester derivatives in acetonitrile containing *N,N′*-diisopropylethylamine. Structure and proposed fragmentation, elution profile, and NICI mass spectra of the (**A**) 2-ClHA and (**B**) 2-ClHyA PFB ester derivatives are shown in the upper, middle, and lower panels, respectively.

can aggravate neuronal dysfunction [5]. Many of the pathways that interfere with BBB and neuronal function converge on the formation of reactive species [6]. This is of importance since TJ proteins are sensitive to alterations of the intracellular redox status [7] and oxidative stress induces a downregulation of the TJ protein occludin and disrupts the cadherin-catenin complex in brain endothelial cells [8]. In cerebrovascular diseases and stroke reactive oxygen species (ROS) can inhibit cerebral blood flow and impact barrier function [9–12]. Additionally, oxidative stress-induced activation of matrix metalloproteinases (MMPs) and fluid channel aquaporins promote leakiness of the BBB and vascular edema [13].

During earlier work we could show pronounced BMVEC barrier dysfunction in response to the chlorinated fatty aldehyde 2-chlorohexadecanal (2-ClHDA) that is generated during endotoxemia [14,15]. 2-ClHDA is formed through attack of plasmalogens (ether phospholipids) by hypochlorous acid/hypochlorite ($HOCl/OCl^-$) [16,17] generated via the myeloperoxidase (MPO)-H_2O_2-Cl^- system of activated phagocytes [18]. Under physiological conditions MPO is part of the innate immune system [19], however, under chronic inflammatory conditions MPO is considered a disease modifier [20]. MPO-derived oxidants have been shown to contribute to atherosclerosis and plaque instability [21–23], cardiac dysfunction [24], or diseases with a neuroinflammatory component [25]. The involvement of MPO in barrier dysfunction was also demonstrated during bacterial meningitis [26,27]. MPO is expressed in demyelinated lesions in Multiple Sclerosis (MS) in humans and rodents [28]. In line, pharmacological inhibition of MPO reduced the severity of clinical symptoms in a murine MS model [29]. In response to systemic lipopolysaccharide (LPS) administration MPO levels in mouse brain are elevated and accompanied by decreased brain plasmalogen content and concomitant formation of 2-ClHDA [14]. In line with deleterious effects of MPO-generated 2-ClHDA [15], the MPO inhibitor *N*-acetyl lysyltyrosylcysteine amide ameliorates brain damage in a murine model of stroke [30] and counteracts BBB damage in a murine model of MS [31].

The electrophile 2-ClHDA impairs protein function by covalent modification, thereby triggering cytotoxic and adaptive responses that are typically associated with oxidative stress [32]. Consequently, conversion of (reactive) aldehydes to their corresponding alcohol and/or carboxylic acid analogues via the fatty alcohol cycle was considered as a protective pathway [33]. The Ford group has first demonstrated that