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History of cigarette smoking in cognitively-normal elders is associated with elevated cerebrospinal fluid biomarkers of oxidative stress

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ABSTRACT

Background: Cigarette smoking in adults is associated with abnormalities in brain neurobiology. Smoking-induced central nervous system oxidative stress (OxS) is a potential mechanism associated with these abnormalities. The goal of this study was to compare cognitively-normal elders on cerebrospinal fluid (CSF) levels of F_2-isoprostane biomarkers of OxS.
Methods: Elders with a lifetime history of smoking (smokers; $n = 50$; 75 ± 5 years of age; 34 ± 28 pack-years; approximately 12% were actively smoking at the time of study) were compared to never-smokers ($n = 61$; 76 ± 6 years of age) on CSF $iPF_{2\alpha}$-III and 8,12, *iso*-$iPF_{2\alpha}$-VI F_2-isoprostanes levels. F_2-isoprostanes levels were quantitated with HPLC-atmospheric pressure chemical ionization-tandem mass spectrometry. Associations between F_2-isoprostanes levels, hippocampal volumes, and cigarette exposure measures were also evaluated.
Results: Smokers showed higher $iPF_{2\alpha}$-III level than never-smokers. An age × smoking status interaction was observed for 8,12, *iso*-$iPF_{2\alpha}$-VI, where smokers demonstrate a significantly greater concentration with increasing age than never-smokers. In smokers only, higher 8,12, *iso*-$iPF_{2\alpha}$-VI concentration was associated with smaller hippocampal volume, and greater $iPF_{2\alpha}$-III level was related to greater pack years.
Conclusions: This is the first study to demonstrate that a history of cigarette smoking in cognitively-normal elders was associated with significantly elevated CSF F_2-isoprostane levels and greater age-related increases in F_2-isoprostanes, and that higher F_2-isoprostane levels in smokers were related to smaller hippocampal volume. These findings provide additional novel evidence that a history of chronic smoking during adulthood is associated with adverse effects on the human brain that are potentially enduring even with extended smoking cessation.

1. Introduction

It is now apparent that cigarette smoking-related morbidity extends well beyond cardiovascular disease, chronic obstructive pulmonary diseases, and various cancers, and includes neurobiological and neurocognitive abnormalities, some of which are progressive over time, and are not directly attributable to the foregoing biomedical conditions (Azizian et al., 2009; Durazzo et al., 2010; Sharma and Brody, 2009; Swan and Lessov-Schlaggar, 2007). Specifically, chronic cigarette smoking in young-to-elder adults, without a history of clinically significant biomedical or psychiatric disorders, is associated with abnormalities in brain morphology, biochemistry, microstructural integrity, and neurocognition (Durazzo et al., 2012, 2013; Kuhn et al., 2012, 2010; Wagner et al., 2013; see Durazzo et al., 2010 for review). Additionally, former-smokers show neurobiological and neurocognitive abnormalities

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that are intermediate to active-smokers and never-smokers (Durazzo et al., 2010). Smoking is also associated with significantly increased risk factor for Alzheimer's disease (AD) and other diseases that promote dementia (Cataldo et al., 2010; Giunta et al., 2012; Peters et al., 2008). Cigarette smoke is a complex mixture of approximately 5000 combustion products that contains a high number of toxic and carcinogenic compounds (Talhout et al., 2011). Chronic cerebral oxidative stress (OxS) is induced by cigarette smoking, and OxS has been suggested as a mechanism promoting the neurobiological and neurocognitive deficits observed in smokers (Durazzo and Meyerhoff, 2007; Fowles et al., 2000; Haustein, 1999; Swan and Lessov-Schlaggar, 2007; Yang and Liu, 2003). Cerebral OxS is operationalized as the detection of damage to brain tissue (e.g., lipid peroxidation, proteolysis) that is caused by reactive oxygen species (ROS), or more broadly by damage from ROS, reactive nitrogen species (RNS), and other oxidizing agents (Seet et al., 2011; Sutherland et al., 2013; Wang and Michaelis, 2010). Increased levels of free radicals and oxidants results from an imbalance between the generation of these compounds by endogenous (i.e., normal cellular metabolism) and/or exogenous sources (e.g., smoking), and their chemical reduction by antioxidants/radical scavengers (Schulz et al., 2000; Valavanidis et al., 2009; Wang and Michaelis, 2010). Cigarette smoke contains extremely high concentrations of short-and-long-lived free radicals (Ambrose and Barua, 2004; Valavanidis et al., 2009), and smoking inhibits synthesis of essential endogenous intracellular anti-oxidants, such as glutathione (Bloomer, 2007; Moriarty et al., 2003). The brain is highly susceptible to OxS damage due to its high metabolism and susceptibility of membrane phospholipids to radical attack (Anbarasi et al., 2006; Chalela et al., 2001; Kovacic, 2005; Mueller et al., 2001), and the hippocampi are particularly vulnerable to OxS (Wang and Michaelis, 2010). Central nervous system (CNS) OxS may trigger inflammation via increased proinflammatory cytokine release (Volobouева and Giffard, 2011), which, in turn, amplifies OxS through generation of additional ROS and other inflammatory mediators (Crews et al., 2006; Guerri and Pascual, 2010; Perricone et al., 2009). Correspondingly, CNS OxS and inflammation tend to occur in tandem in many diseases/disorders, including AD, atherosclerosis, alcohol/substance use disorders, and cigarette smoking (Butterfield et al., 2013; Crews and Nixon, 2009; Durazzo et al., 2010; Enciu et al., 2013; Gill et al., 2010; Khandelwal et al., 2011; Khanna et al., 2013). Animal models and human post-mortem studies indicate that cigarette smoking-induced OxS promotes brain tissue damage via lipid peroxidation and proteolysis (Anbarasi et al., 2005, 2006; Ho et al., 2012; Khanna et al., 2013; Rueff-Barroso et al., 2012; Sonnen et al., 2009; Tyas et al., 2003). Since smoking is a risk factor for AD, it is possible that smoking-induced OxS increases the risk for AD-like neuropathological changes; however, there are no published *in vivo* studies that have specifically compared human smokers and non-smokers on CNS-derived biomarkers of cerebral OxS.

The goal of this study was to compare cognitively-normal elders with a history of cigarette smoking during lifetime (smokers) to never-smokers on cerebrospinal fluid (CSF) levels of F_2-isoprostanes, and test the associations between these biomarkers of OxS and hippocampal volume, a structure that typically shows marked atrophy in those with mild cognitive impairment and AD (Jack et al., 2013). F_2-isoprostanes are prostaglandin-like compounds formed from free radical-mediated peroxidation of arachidonic acid, a highly abundant polyunsaturated fatty acid in the brain (Korecka et al., 2010; Milne et al., 2005). F_2-isoprostanes are established biomarkers of radical-induced OxS (Rokach et al., 2004) that have been employed to assess OxS-related tissue damage in neurodegenerative diseases, atherosclerosis, pulmonary diseases, and chronic smoking (Galasko and Montine, 2010; Korecka et al., 2010; Milne et al., 2005; Pratico, 2008, 2010; Yao et al., 2003), and F2-isoprostane levels increase with advancing age (Montine et al., 2011). In this report we adhere to the F_2-isoprostane nomenclature proposed by Rokach et al. (1997). $iPF_{2\alpha}$-III (alternate nomenclatures: 8-*iso*-$PGF_{2\alpha}$; 15-F_{2t}-IsoP) and 8,12, *iso*-$iPF_{2\alpha}$-VI (alternate nomenclature: 5-F_{2c}-IsoP) are among the most studied of the F_2-isoprostanes, with the majority of research focused on $iPF_{2\alpha}$-III (Cracowski et al., 2002; Haschke et al., 2007; Korecka et al., 2010). In active and former adult smokers, urine and plasma $iPF_{2\alpha}$-III levels were significantly elevated relative to never-smokers (Harman et al., 2003; Helmersson et al., 2005; Milne et al., 2005; Yan et al., 2007). CSF F_2-isoprostane levels may more accurately characterize lipid peroxidation of brain tissue than those obtained from peripheral levels because blood and urine-based F_2-isoprostanes concentrations will also reflect radical-mediated peroxidation of plasma lipids and tissue comprising peripheral organ systems (Milne et al., 2005; Montine et al., 2011). We posit that a history of cigarette smoking serves as a source of chronic OxS that places a significant burden on the integrity of brain morphology, which is exacerbated by advancing age. Accordingly, we tested the following hypotheses:

1. Smokers demonstrate significantly higher CSF $iPF_{2\alpha}$-III and 8,12, *iso*-$iPF_{2\alpha}$-VI and (*iso*-$iPF_{2\alpha}$-VI) concentrations than never-smokers, and a smoking status (smoker vs. never-smoker) × age interaction is observed, where smokers show significantly higher $iPF_{2\alpha}$-III and *iso*-$iPF_{2\alpha}$-VI levels with increasing age than never-smokers.
2. In smokers and never-smokers, higher CSF $iPF_{2\alpha}$-III and *iso*-$iPF_{2\alpha}$-VI concentrations are associated with smaller hippocampal volumes.
3. In smokers, higher cigarette pack-years are related to higher CSF $iPF_{2\alpha}$-III and *iso*-$iPF_{2\alpha}$-VI concentrations.

2. Methods

2.1. Participants and study design

Participants were 111 cognitively-normal elder controls (75.5 ± 5.1 years of age) from Alzheimer's disease Neuroimaging Initiative (ADNI) project, Phase 1. Phase 1 of ADNI (ADNI1) was a multisite study supported by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the FDA, private pharmaceutical companies, and non-profit organizations, as a 5-year public–private partnership. The primary goal of ADNI1 was to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biomarkers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD (Mueller et al., 2005a, 2005b). Written informed consent was obtained from all participants before procedures were performed. The study was conducted according to the Declaration of Helsinki, and U.S. 21 CFR Part 50–Protection of Human Subjects, and Part 56–Institutional Review Boards.

Participants who reported they never smoked cigarettes during lifetime were assigned to the never-smoker group ($n = 61$), and those who reported any history of cigarette smoking during lifetime were designated as smokers ($n = 50$). Twenty-five of 50 smokers had sufficiently detailed smoking history information to calculate pack-years (34 ± 28); three participants were actively smoking at the time of study, and 22 were former-smokers with 35 ± 14 years of smoking cessation. Antioxidant (vitamin E), anti-hypertensive, and statin/cholesterol absorption blocking agents (statin/CAB) usage was recorded (binary variables—yes, no) and body mass index (BMI) calculated for all participants. See Table 1 for group demographic and clinical information.

2.2. CSF $iPF_{2\alpha}$-III and iso-$iPF_{2\alpha}$-VI acquisition and quantitation

ADNI procedures for CSF sampling via lumbar puncture, transport, and storage were previously described in detail (Shaw et al., 2009). Quantitation of $iPF_{2\alpha}$-III and *iso*-$iPF_{2\alpha}$-VI was achieved with a HPLC-atmospheric pressure chemical ionization-tandem mass spectrometry method that demonstrates high sensitivity and selectivity for these F_2-isoprostanes (Korecka et al., 2010).

2.3. MRI image acquisition and processing

Participants completed a 1.5 T magnetic resonance scan. T1-weighted MRI scans using 3D volumetric magnetization prepared rapid gradient echo (MPRAGE) and 3D T2-weighted sequences were acquired for morphological analyses (see Jack et al., 2008 for acquisition parameters). All images were calibrated with phantom-based