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Review

Low-dose aspirin (acetylsalicylate) prevents increases in brain PGE_2, 15-epi-lipoxin A4 and 8-isoprostane concentrations in 9 month-old HIV-1 transgenic rats, a model for HIV-1 associated neurocognitive disorders

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ABSTRACT

Background: Older human immunodeficiency virus (HIV)-1 transgenic rats are a model for HIV-1 associated neurocognitive disorders (HAND). They show behavioral changes, neuroinflammation, neuronal loss, and increased brain arachidonic acid (AA) enzymes. Aspirin (acetylsalicylate, ASA) inhibits AA oxidation by cyclooxygenase (COX)-1 and COX-2.
Hypothesis: Chronic low-dose ASA will downregulate brain AA metabolism in HIV-1 transgenic rats.
Methods: Nine month-old HIV-1 transgenic and wildtype rats were given 42 days of 10 mg/kg/day ASA or nothing in drinking water; eicosanoids were measured using ELISAs on microwaved brain extracts.
Results: Brain 15-epi-lipoxin A4 and 8-isoprostane concentrations were significantly higher in HIV-1 transgenic than wildtype rats; these differences were prevented by ASA. ASA reduced prostaglandin E_2 and leukotriene B_4 concentrations in HIV-1 Tg but not wildtype rats. Thromboxane B_2, 15-HETE, lipoxin A_4 and resolvin D_1 concentrations were unaffected by genotype or treatment.
Conclusion: Chronic low-dose ASA reduces AA-metabolite markers of neuroinflammation and oxidative stress in a rat model for HAND.

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1. Introduction

Human immunodeficiency virus (HIV)-1-infected patients are at risk of developing HIV-1 associated neurocognitive disorders (HAND) [1,2]. These disorders progress over time, ranking from mild neurocognitive impairment to HIV-1-associated dementia, which involves severe cognitive dysfunction in multiple domains [3]. The introduction of antiretroviral therapy (ART) has reduced the prevalence of dementia. However, as the lifespan of HIV-1 infected patients has been prolonged by ART, the prevalence of HAND with aging remains high [4].

HAND probably arises from direct and maintained viral invasion of the central nervous system (CNS), which is an important reservoir for HIV-1 virus regardless of plasma viral suppression or cumulative time on ART. In the North-East AIDS Dementia consortium, over 50% of viremically controlled HAND patients had detectable virus in the cerebrospinal fluid (CSF) [5]. Aberrant macrophage and T-lymphocyte activation in the CSF continued despite viremic control by ART [6], and brain atrophy correlated with the CSF level of quinolinic acid, evidence of activated CNS macrophages and microglia associated with neuroinflammation and excitotoxicity [7].

HIV-1 infection of the brain stimulates both the innate and adaptive immune systems. Brain damage and neuronal loss are associated with activation of microglia, astrocytes and invasive macrophages, which release toxic quantities of agents such as nitric oxide, tumor necrosis factor (TNF)-α, interleukin (IL)-6, and interleukin (Il)-1β [8]. Additionally, preclinical evidence indicates that there is secondary activation of phospholipases A_2 (PLA_2) that release the n-6 polyunsaturated fatty acid arachidonic acid (AA)

Abbreviations: AA, arachidonic acid; ART, antiretroviral therapy; ASA, acetylsalicylic acid; CNS, central nervous system; COX, cyclooxygenase; CSF, cerebrospinal fluid; DHA, docosahexaenoic acid; HAND, HIV-1 associated neurocognitive disorders; HETE, hydroxyeicosatetraenoic acid; HIV, human immunodeficiency virus; LOX, lipoxygenase; LT, leukotriene; LX, lipoxin; PG, prostaglandin; PLA, phospholipase; TX, thromboxane

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from membrane phospholipids [9]. AA and many of its eicosanoid products are proinflammatory. They include prostaglandin (PG)E_2 formed preferentially by cyclooxygenase (COX)-2, thromboxane (TX)B_2 formed preferentially by COX-1, 15(*S*)-HETE formed by 15-lipoxygenase (LOX), lipoxin (LX)A_4 formed from 15-HETE by 5-LOX [10], leukotriene (LT)B_4 also derived from AA by 5-LOX [11], 15-epi-LXA_4 synthesized from 15(*R*)-HETE by acetylated COX-2 [12,13], and isoprostanes produced by non-enzymatic pathways [14,15]. COX-2 expression is increased following injection of HIV-1 Tat or gp-120 proteins into rodent brain, and in other HIV-1 rodent models [16–20].

Evidence for neuroinflammation in HIV-1 patients, and the recognized relation between neuroinflammation and upregulated AA metabolism in animal HIV-1 models and human neurodegenerative disease [21–23], suggest that antiinflammatory drugs that target the brain AA cascade might be of clinical relevance. One such drug is aspirin (ASA, acetylsalicylic acid), whose antiinflammatory actions in peripheral inflammation are well described [24,25]. ASA inhibits COX-1, which converts AA to TXB_2, while acetylating and inhibiting COX-2, which converts AA to PGE_2 and also becomes capable of converting docosahexaenoic acid (DHA) to antiinflammatory 17*R*-hydroxy-containing di- and tri-hydroxy-docosanoids termed resolvins [26]. Evidence in animals and humans indicates that even low dose aspirin can exert behavioral and other biological effects in the intact CNS [27–32].

To provide a basis for testing potential efficacy of ASA in HIV-1 patients with HAND, we examined in the present study the effect of low-dose ASA on brain AA and DHA metabolism in a transgenic (Tg) rodent model of HIV-1, and in control rats. The human HIV-1 provirus, carrying seven of the nine HIV-1 genes after functional deletion of the infectious genes *Gag* and *Pol* is constitutively expressed by the HIV-1 Tg rat. The HIV-1 Tg rat develops neuropathology as it ages, thus may be an animal model for HAND [33,34]. It shows reduced spatial learning at 5 months of age. At 7–9 months, neuroinflammation and synaptic loss occur, associated with increased expression of AA-metabolizing cytosolic $cPLA_2$ IVA, secretory $sPLA_2$ IIA and COX-2 [19] and changes in fatty acid composition [35].

We treated 9-month old HIV-1 Tg and wildtype rats with 10 mg/kg/day ASA in drinking water for 42 days, using ASA-free water as a control. This ASA regimen is equivalent to a low therapeutic dose of 100 mg in human (for a 70 kg subject) [36–38]. We used ELISA assays to measure brain concentrations of PGE_2, thromboxane TXB_2, leukotriene LTB_4, lipoxin LXA_4, 15-epi-LXA_4, 15-hydroxyeicosatetraenoic acid (HETE), 8-isoprostane and resolvin D1.

2. Materials and methods

2.1. Chemicals

ASA was purchased from Sigma-Aldrich (Saint Louis, MO). Hexane and isopropanol (Reagent Grade) were obtained from Fisher Scientific (Pittsburgh, PA). Ultra-pure water was purchased from KD Medical (Columbia, MD).

2.2. Animals

The experiments were conducted under an approved NICHD animal protocol (12–027) in accordance with the NIH Guidelines on the Care and Use of Laboratory Animals. Age-matched male HIV-1 Tg or Fischer 344/NHsd wildtype rats (9 months-old), purchased from Harlan Laboratories (Madison, WI) were housed in an animal facility under a 12 h/12 h light–dark cycle with ad libitum access to water and an identical Teklad global 18% protein 2018*S* diet. The diet was 2018*S* (sterilized) for controls and 2918 (irradiated 2018*S*) for HIV-1 Tg rats (Teklad Harlan, Madison, WI). The diets were processed in identical ways, except that the 2018*S* diet was further gamma irradiated to minimize the risk of infection in the HIV-1 Tg colony. The diet contained (as % of total fatty acid) 16.7% saturated, 21.8% monounsaturated, 54.8% linoleic acid, 6.2% α-linolenic acid, 0.03% AA, 0.02% eicosapentaenoic (EPA, 20:5 n-3) and 0.06% docosahexaenoic acid (DHA, 22:6 n-3) [35]. After 42 days on the diet, the rats were anesthetized with Nembutal (40 mg/kg, i.p.), and subjected to head-focused microwave irradiation at 5.5 kW for 3.4 s (Cober Electronics, Stamford, CT). The brain was immediately removed, placed on dry ice, and stored at −80 °C.

2.3. ASA treatment

Before going on the diet, rats were separated into four different groups ($n=12$): untreated wildtype, untreated HIV-1 Tg, ASA-treated wildtype, and ASA-treated HIV-1 Tg. Untreated groups received regular drinking water. The ASA-treated groups received ASA in water (10 mg/kg/day) for 42 days. Fresh ASA drinking water was prepared and provided every two days, as was control drinking water. Rat weight and water intake were monitored weekly. According to an interspecies conversion factor based on body surface, the 10 mg/kg/day dose used here was equivalent to a dose of 100 mg for a 70 kg person [36].

2.4. Sample preparation

Extraction of oxygenated metabolites of AA and DHA was performed according to the Radin method [39]. Half-brains were homogenized in a glass Tenbroeck homogenizer in hexane–isopropanol (3:2 v-v, 18 ml/g brain). The homogenate was transferred to a glass centrifuge tube and the homogenizer was washed twice with 4 volumes of hexane–isopropanol solution. The pooled homogenate was centrifuged at 1500 rpm for 5 min at room temperature, and the organic supernatant was collected. The pellet was re-extracted twice in 5 ml hexane–isopropanol. The pooled extracts were dried under N_2 at 45 °C, resuspended in 3 ml hexane–isopropanol and stored at −80 °C.

2.5. Measurement of brain eicosanoids and docosanoids by enzyme immunoassay

To perform an enzyme immunoassay, 1 ml of the sample in hexane–isopropanol was dried under N_2 and resuspended in 500 μl buffer. Concentrations of eicosanoids or docosanoids were determined with commercially available ELISA kits in accordance with the manufacturer's instructions. PGE_2 and 15-epi-LXA_4 kits were obtained from Oxford Biochemical (Oxford, MI) and TXB_2, LTB_4, 15-HETE, LXA_4, 8-isoprostane and Resolvin D1 kits were from Cayman Chemicals (Ann Arbor, MI).

2.6. Statistical analyses

All data are expressed as mean ± SEM ($n=10$–12 per group). In some groups, an outlier was identified using Grubbs' test, and removed from the data set (as indicated in the figure legend). For 8-isoprostane quantification, $n=10$ for controls and $n=11$ for HIV-1 Tg rats due to sample shortage. A two-way ANOVA was performed to identify global effects of genotype and treatment (GraphPad Prism 5.0, GraphPad Software, La Jolla, CA), and was followed by Least Significant Difference (LSD) post-hoc tests for multiple comparison. The level of significance was set at $p<0.05$.