

Alzforum News Highlights: Caffeine, Anesthesia, and Twin Epigenetics

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CAFFEINE RUSH? REVERSING COGNITIVE WOES, A β LOAD in AD MICE

16 July 2009. Could the morning coffee that provided a lift for the day also help you stay mentally sharp for the long haul? This prospect, which has simmered on the backburner of neurodegenerative disease research, has bubbled to the fore with the publication of two mouse studies in this month's *Journal of Alzheimer's Disease* [1,2]. Led by Gary Arendash, researchers at the University of South Florida, Tampa, with collaborators elsewhere, report that caffeine restores normal cognition and decreases brain and blood amyloid- β (A β) levels in old, memory-impaired Alzheimer's disease (AD) mice. The work also sheds light mechanistically on how caffeine suppresses β - and γ -secretases, enzymes that help churn out the peptides in the pathological plaques peppering AD brains.

At the end of the year, the *Journal of Alzheimer's Disease* will publish a focus issue featuring these and other studies presented at a small, closed meeting, "Caffeine and the Brain," held in Lisbon last month.

The idea that caffeine could protect against AD has percolated for some time, through both epidemiological studies [3] and research in animal models. In 2002, a case-control study by Portuguese researchers found that daily caffeine consumption by AD patients in the 20 years preceding disease onset was much lower than that of same-aged participants who did not develop AD [4]. Earlier this year, scientists reported that people who drank three to five cups of coffee per day during midlife had a 65 percent reduced risk for AD in a longitudinal study of more than 1,400 Finnish seniors [5]. On the basic science front, Gary Arendash and colleagues at

the University of South Florida have tackled the issue with a controlled longitudinal study in AD transgenic mice (A β PPsw). They provided caffeine-spiked drinking water (1.5 mg per day, equivalent to five cups of coffee in humans) to four-month-old young adult mice and continued the treatment through nine months of age, when the mice typically have AD-like behavioral symptoms. When tested at this older age, the caffeinated AD mice performed virtually as well as non-transgenic controls in a host of cognitive tasks, and had reduced brain amyloid load compared with untreated AD mice. In that study [6], caffeine's benefits seemed to stem from its suppression of β - and γ -secretases. "That was our initial indication that it was having a direct effect on the disease process and not simply acting as an anti-inflammatory or something that just affects attention or reaction time," Arendash said in an interview with the *Alzheimer Research Forum* (ARF).

His team designed the current study to further unpack caffeine's mechanism of action and to determine whether its benefits could extend to older AD mice that already show AD-like pathology and cognitive problems. To address the latter question, Arendash and colleagues withheld caffeine administration in the same AD transgenic mice (A β PPsw) until 19 months of age, when the mice had memory impairment. After five weeks of treatment at the same daily dose used previously (1.5 mg), the impaired AD mice greatly improved their performance on the radial arm water maze, a common rodent test of working memory, compared to transgenic counterparts chugging regular water. Like the prior study that tested caffeine as a preventive treatment, caffeine consumption in older, impaired mice restored their cognitive performance to levels indistin-

guishable from same-aged wild-type animals. Furthermore, the caffeine treatment strikingly reduced A β deposition and soluble A β levels in the entorhinal cortex and hippocampus of treated relative to untreated AD mice.

Delving further into the mechanisms underlying these effects, the scientists found that caffeine's suppression of β -secretase involves toning down the Raf-1/NF- κ B inflammatory pathway. Their results also suggest that caffeine suppresses γ -secretase by inhibiting its primary stimulator, glycogen synthase kinase (GSK)-3 α . There could be a tie-in with tau, too, since GSK-3 β , a key stimulator of neurofibrillary tangle (NFT) formation, was also suppressed by caffeine in the recent study. "I would be very surprised if caffeine did not suppress NFT formation or tau phosphorylation, but we have not looked at that specifically yet," Arendash told ARF.

In the meantime, the scientists are excited about caffeine's effects on A β load, which are not only behaviorally meaningful but also fast. In the same issue of the *Journal of Alzheimer's Disease*, a study led by Chuanhai Cao, also at the University of South Florida, and collaborator David Holtzman at Washington University School of Medicine in St. Louis, Missouri, shows that acute caffeine administration reduces both brain and blood A β levels in AD transgenic mice within a few hours [2]. Arendash said his group has found similar effects in unpublished human studies involving acute caffeine administration. "The fact that we can get a caffeine response within hours in AD mice and in humans, to me, is encouraging," he said.

To address the possibility that caffeine might have had some effects on mice through non-AD-specific mechanisms, Arendash and colleagues gave wild-type mice caffeinated drinking water throughout adulthood and examined their cognition in older age. The caffeinated mice fared no better than untreated counterparts on the radial arm water maze and four other cognitive tasks. "We believe caffeine only affects memory when it is impaired or is going to be impaired," Arendash said. "It's not going to be a memory-enhancing drug over and above normal."

Still, Arendash thinks caffeine holds promise as an inexpensive, brain-penetrating agent that warrants long-term clinical trials in AD patients. It meets all three criteria he deems critical for a successful AD therapeutic: 1) strong epidemiological evidence for effectiveness; 2) compelling behavioral results in AD mice; and 3) demonstrated effects on disease process. "If you have all three of those lined up, at least in 2009, you

have maximized your chances of seeing a therapeutic effect against the disease in humans," Arendash said. "Our whole concept of how to pursue an effective therapeutic against AD in the shortest amount of time is to focus on therapeutics that are inherently safe or that are already in use clinically for other sorts of medical issues. When you focus on therapeutics like that, you are cutting years off drug development."

In the spirit of disclosure, Arendash noted that both caffeine studies were financed by the NIH-designated Florida Alzheimer's Disease Research Center, directed by Huntington Potter, and not by any coffee industries. And on a lighter note, he practices what he preaches. "I've been drinking coffee, at least three to four cups a day, ever since I can remember. I think perhaps I've increased it a little bit after our studies showing that five cups seems optimal. But yeah, I've had four cups today. I'm right on schedule," he quipped during a recent phone conversation with this reporter. – Esther Landhuis.

INHALING ALZHEIMER'S? HAZY PICTURE LINKS ANESTHESIA, AD

31 July 2009. Anesthesia is meant to induce a temporary fog, lifting as soon as the drugs wear off. But for some, the effects linger, with delirium or confusion for days or weeks following surgery. Over the last decade, a small cadre of scientists has begun investigating whether anesthesia might occasionally have permanent effects, pushing some people closer to AD [7]. Although cell culture and animal experiments show common anesthetics can cause A β production and apoptosis, the possible connection in people is hazier. "We have a smoking gun, but no victim at this point," said Roderic Ekenhoff, an anesthesiologist who researches the topic at the University of Pennsylvania in Philadelphia.

Scattered case reports of mental fuzziness after surgery have been part of the scientific literature for years, and doctors occasionally hear of a patient who was never quite the same, cognitively, after a spell on the operating table. "That story is so common," Ekenhoff said. "We give these drugs to tens of millions of patients every year, and blithely ignore that they could have long-term effects." At a recent workshop in Philadelphia, Ekenhoff and others concluded that the current data on anesthesia and AD are worrisome enough to warrant further studies [8]. Researchers will also convene this fall to plan multi-site clinical studies.

With clinical data scarce, researchers say it is too soon to alter operating room practice. But a few are already modifying their treatment of elderly patients. “I’m telling my own family and friends to avoid isoflurane,” said Rudy Tanzi of the Massachusetts General Hospital in Boston, referring to a common inhalation anesthetic. Tanzi’s mother had several surgeries including hip and knee replacements when she was in her early seventies. She always experienced a few days of mild delirium following an operation – until the most recent surgery, when Tanzi asked the anesthesiologist to swap isoflurane for desflurane. Following that procedure, his mother awoke clearheaded, “like nothing happened,” Tanzi recalled. When she heard it was 7:30, she demanded he turn on the TV because the Red Sox were playing and she wanted to see David “Big Papi” Ortiz. “It’s an n of one, but it’s my mother, so it counts for a lot,” Tanzi said.

In vitro and animal studies link common anesthetics to both the A β plaques and tau tangles found in AD. Tanzi and Zhongcong Xie of Massachusetts General Hospital in Boston have shown that inhaled compounds such as isoflurane increase production of A β in cultured cells [9,10], and Eckenhoff and colleagues found that the same chemicals lower the concentration of A β needed to oligomerize in a test tube [11,12]. Emmanuel Planel of Laval University in Québec City has found that anesthetics and the accompanying hypothermia can increase phosphorylation of tau, too [13].

Anesthesia also causes apoptosis of neurons, and the apoptotic pathways and A β can feed off of each other in a vicious cycle. Caspases, activated in apoptosis, destroy the chaperone GGA3, which normally restricts BACE activity. Unchecked, BACE cleaves more A β PP, setting it on the path to become A β [14]. At the same time, the addition of A β to cells increases caspase activity further [15].

Based on the cell and animal data, scientists hypothesize that similar damage might occur in the human brain. For a cognitively normal person, the effects of anesthesia may be minimal. But for a person already at risk for AD – maybe because of an apolipoprotein E4 (ApoE4) genotype or mild cognitive impairment – anesthesia may be the final insult that launches the brain into AD mode. “It’s like a stepping stone,” said Walter Schulz-Schaeffer of the University of Göttingen in Germany. In animals, too, those most prone to AD-like pathology may be most sensitive to the effects of anesthesia. For example, in Planel’s tau experiments, wild-type mice quickly return to normal levels of tau phosphorylation after anesthesia, but animals carrying

a tau mutation associated with frontotemporal dementia do not [16]. However, researchers emphasize that without human studies, their ideas remain unproven.

Part of the challenge is that despite more than a century of experience with anesthetics, doctors still do not know exactly how the drugs work [17]. Inhaled anesthetics interact with enzymes, receptors, transporters, channels, and structural proteins. At least some of the damaging effects of anesthesia may be due to the hypothermia that comes with it. Anesthesia throws off the body’s thermostat: “Patients become, for lack of a better term, cold-blooded during surgery,” Eckenhoff said. In Planel’s studies of tau under anesthesia, maintaining normal temperature abolished the increased tau phosphorylation that anesthesia usually produced. Keeping patients warm is an easy precaution that doctors can take now, Planel said: “It wouldn’t hurt.”

And temperature is just one factor that doctors should track so that they can analyze the effects of anesthesia. A surgical stay in the hospital will also involve pain, stress, inflammation, perhaps infection, all of which could confound studies of cognition. A person may have cognitive impairment before an operation, but friends and family might not notice until afterward, and mistakenly attribute the problem to the surgery. To truly sort out the relationship between AD and anesthesia will require a large, expensive study.

Clinical research has already confirmed the immediate cognitive effects of surgery, which fit two categories. Acute post-operative delirium (POD), in which patients may not know who or where they are, may last a few days. Post-operative cognitive dysfunction (POCD), causing people to struggle with basic tasks such as memory and word-finding, may not dissipate for weeks or months. Some scientists suspect these conditions are short-term variants of AD-like pathology, perhaps caused by a temporary A β increase that the brain eventually overcomes. Even a single episode of delirium is associated with quickened cognitive decline in people who already have AD [18]. But the relationship is tricky to study, Xie said, because data are missing on both sides. Doctors know POD and POCD are real, but have no information on the underlying molecular mechanisms. Researchers have used mazes to test rodents for the effects of POCD on memory and learning [19–21], but delirium is harder to assess in animal models. “How do you know if a mouse has lost touch with reality?” Eckenhoff asks. Conversely, scientists know plenty about the neuropathology of AD, but lack positive proof that it is associated with anesthesia.

Xie has commenced a couple of studies to look for anesthesia effects in people. In one project, he is ex-

aming brain tissue removed during surgery to treat epilepsy, looking for evidence of apoptosis markers, enzymes involved in $A\beta$ processing, and phosphorylated tau. Xie and Eckenhoff are collaborating in a study of cerebrospinal fluid samples, collected when people undergo spinal anesthesia. They hope to correlate levels of $A\beta$ in cerebrospinal fluid with the severity of post-operative cognitive symptoms.

Should scientists conclusively link anesthesia to AD or $A\beta$, they could then look for ways to circumvent the problem. Anesthesiologists might screen for vulnerable people who need special care or should avoid certain anesthetics. For instance, one study linked the ApoE4 allele to a predisposition for POD [22]. Scientists could also look for co-treatments that might diminish anesthesia's impact on cognition. Tanzi and Xie found that the AD drug memantine reduced the caspase activation caused by isoflurane in a cell culture study [23]. Planel is also testing memantine to see if it attenuates the pathology he sees in anesthetized mice.

At this point, doctors and patients have precious little information to go on. Xie regularly fields e-mails from concerned people facing surgery, and he tells them to follow their anesthesiologist's recommendations, and not to base medical decisions on limited laboratory results. "If you try another drug, how do you know the other drug is not also dangerous?" he asked. "Simply, we do not know the answers." – Amber Dance.

TWIN STUDY SUGGESTS EPIGENETIC DIFFERENCES in AD

21 August 2009. As is true with getting into college or receiving a job offer, it's not just pedigree but also life experiences that may determine whether a person will develop AD. So suggests an analysis of identical twins – one who died of AD, one without AD – reported this month in the publicly accessible journal PLoS ONE [24]. Researchers led by Paul Coleman, Sun Health Research Institute, Sun City, Arizona, examined postmortem brain tissue and found that cortical neurons from the AD twin had reduced DNA methylation, a biochemical process that can disrupt genes' accessibility for transcription by attaching methyl groups to individual nucleotides.

In an earlier study [25], first author Diego Mastroeni and colleagues found lower levels of DNA methylation, as well as reduced expression of DNA methyltransferase and other methylation regulators, in affected brain areas of sporadic AD patients. "This led to

the question of whether these epigenetic effects we saw in AD were related to the [people's] genes or to their life experience," said Coleman, who is also a professor emeritus at the University of Rochester Medical Center, New York. In the sporadic AD study, genetic backgrounds were all over the map – which is why the scientists leaped at the opportunity to analyze epigenetic markers in identical twins discordant for AD. "This was a situation in which the genetic background would be quite similar, if not identical, and anything we saw could be attributed to life experience," Coleman said. Other research has shown that identical twins genetically prone to AD can differ markedly in their age of onset and degree of pathology [26].

The twins in the current study were white males who attended the same schools and worked as chemical engineers. One encountered extensive pesticides in his work and died at age 76 after a 16-year battle with AD. The other worked in a different environment and was cognitively normal when he died of prostate cancer at age 79. Pathologically, their brains could not have looked more different. At the time of his death, the twin with AD had an anterior temporal neocortex riddled with amyloid plaques and NFTs, the two key pathological hallmarks of AD. In his non-demented brother, however, "we had to hunt through the brain sections in order to find even one neurofibrillary tangle," Coleman told ARF. The cognitively intact man also had comparatively higher expression of 5-methylcytosine, a marker of methylated cytosine-guanine (CpG) sites on DNA, in neurons, reactive astrocytes, and microglia of brain areas typically vulnerable to AD.

Apart from disease status, DNA methylation appears to vary with age and environmental factors. In a recent analysis of 217 non-pathological human tissues, published this month in PLoS Genetics [27], researchers report that genes in CpG islands become increasingly methylated as people get older, whereas genes outside of these methylation hotspots lose methylation with age. Methylation status also correlated with environmental exposures such as tobacco smoking in that analysis, led by Karl Kelsey at Brown University. In an earlier study, Manel Estrella and colleagues at the Spanish National Cancer Center, Madrid, analyzed identical twins and found that DNA methylation status was very similar when the siblings were young but diverged more and more as they got older [28]. Those papers "make the case for environmental and aging effects on methylation," Coleman said of the Estrella and Kelsey studies. "Our research shows that the concept of life events affecting DNA methylation may apply to devel-

opment of the AD phenotype. It also stresses the potential importance of epigenetic phenomena in molecular mechanisms of AD.”

The new data may have ramifications for interpreting studies of AD genetics. “One study will find that, yes, this gene is a risk factor for AD, and others say, no, it’s not, and the statistics have some uncertainty in them,” Coleman said. “We raise the question of whether the probabilistic nature of the relationship between some genes and AD may be due to the fact that the genetic effects can be modulated by life experience.”

A recent study in Iceland may offer a case in point. Researchers at the University of Iceland and at deCODE Genetics, Reykjavik, reported a drastically shortened lifespan over the last 20 years in people with a hereditary amyloid angiopathy, and attribute this to diet changes that may have exacerbated the effects of a genetic mutation tied to the disease [29]. Studies in AD mouse models that overexpress mutant amyloid precursor protein (TgCRND8 and 129Sv) offer another example of a diet-gene interaction. When put on a diet deficient in folate, B1, and B6, the AD mice had reduced brain methylation activity in conjunction with A β overproduction and cognitive impairment [30].

A link between epigenetics and AD also came up in a recent investigation led by Axel Schumacher at the Klinikum Rechts der Isar, Munich, Germany. However, unlike the current study, which reveals global demethylation in affected brain areas of the AD twin, Schumacher’s showed that most DNA methylation changes in AD brains are subtle and restricted to specific genes, including several involved in A β processing (PSEN1, ApoE) and methylation homeostasis (MTHFR, DNMT1) [31]. In an e-mail to ARF, Schumacher noted that analyzing late-stage disease tissue makes it hard to determine whether the observed epigenetic phenotypes are the cause or the result of the disease. In the new study, “the global demethylation in the affected brain areas may indicate that specific components of the epigenetic machinery, such as DNA maintenance methylation, were inactivated, which in turn could indicate that the observed epigenetic patterns result from the course of the disease,” he wrote.

Coleman hopes to address this possibility in a genomewide study to identify specific genes affected by DNA methylation in AD, he told ARF. Future work in this area may benefit from a new approach that uses flow cytometry and state-of-the-art sequencing techniques to quantify the number of methylated molecules in a sample. Its developers show the method is sensitive enough to detect one methylated molecule in about

approximately 5,000 unmethylated molecules in DNA from plasma or fecal samples. In a report published online 16 August in *Nature Biotechnology* [32], researchers led by Sanford Markowitz, Case Western Reserve University, Cleveland, Ohio, and Bert Vogelstein at Johns Hopkins University School of Medicine, Baltimore, Maryland, have used the technology to detect early-stage colorectal cancer. – Esther Landhuis.

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