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Effect of sleep on overnight CSF amyloid- β kinetics

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Abstract

Sleep disturbances are associated with future risk of Alzheimer's disease. Disrupted sleep increases soluble amyloid- β , suggesting a mechanism for sleep disturbances to increase Alzheimer's disease risk. We tested this response in humans using indwelling lumbar catheters to serially sample cerebrospinal fluid while participants were sleep-deprived, treated with sodium oxybate, or allowed to sleep normally. All participants were infused with ¹³C₆-leucine to measure amyloid- β kinetics. We found that sleep deprivation increased overnight amyloid- β -38, amyloid-

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Brendan P. Lucey, Terry J. Hicks, Jennifer S. McLeland, Cristina D. Toedebusch, Jill Boyd, Jack Baty, John C. Morris: None.

Donald L. Elbert: Dr. Elbert receives royalties from C2N Diagnostics for a patent related to modeling β -amyloid stable isotope labeling kinetics.

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Randall J. Bateman: Washington University, Dr. Bateman, and Dr. David Holtzman have equity ownership interest in C2N Diagnostics and receive royalty income based on technology (stable isotope labeling kinetics and blood plasma assay) licensed by Washington University to C2N Diagnostics. Dr. Bateman receives income from C2N Diagnostics for serving on the scientific advisory board. Washington University, with Dr. Bateman as co-inventor, has submitted the US nonprovisional patent applications "Methods for Measuring the Metabolism of CNS Derived Biomolecules In Vivo" and provisional patent application "Plasma Based Methods for Detecting CNS Amyloid Deposition."

β -40, and amyloid- β -42 levels by 25–30% via increased overnight amyloid- β production relative to sleeping controls. These findings suggest that disrupted sleep increases Alzheimer's disease risk via increased amyloid- β production.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive impairment and neuropathologic extracellular plaques of amyloid- β (A β) and intracellular inclusions of tau. Sleep disturbances are associated with future risk of AD (1–3). For example, women with sleep efficiency <70% have 1.61 higher odds of developing cognitive impairment than women with sleep efficiency 70% or greater (1).

Recent evidence suggests increase risk of AD from sleep disturbances may be due, at least in part, to an A β mechanism. Diurnal oscillation of interstitial fluid (ISF) and cerebrospinal fluid (CSF) A β concentrations in both mice and humans has been replicated in multiple studies using different assays (4–7). A β aggregation as insoluble plaque is concentration-dependent and is hypothesized to be a key early step in AD pathogenesis. Sleep disruption in mice (4) and humans (8, 9) increases soluble A β concentrations and, potentially, plaque deposition. While these findings suggest sleep as a potential novel modulator of AD pathology, we do not know if sleep alters A β production and/or clearance, leaving a critical gap in our ability to pursue sleep modulation as a preventive strategy for AD. In this pilot study, we collected serial CSF from sleep-deprived, sleep-induced, and control participants who were infused with $^{13}\text{C}_6$ -leucine to measure A β stable isotope labeling kinetics (SILK).

Methods

Participants and Sleep Interventions

Eight participants aged 30–60 years old were recruited from both longitudinal studies at the Knight Alzheimer Disease Research Center and a research volunteer registry at Washington University (Volunteers for Health). Three participants were male. All participants were cognitively normal (Clinical Dementia Rating score of 0 or Mini-Mental Status Examination (MMSE) ≥ 27 (10, 11)). Participant characteristics are shown in Table 1. The study was conducted at Washington University School of Medicine in St Louis, Missouri. The study protocol was approved by the Washington University Institutional Review Board and the General Clinical Research Center Advisory Committee. The Clinical Trials number was NCT02063217. All participants completed written informed consent and were compensated for their participation in the study.

Participants were initially randomized to one of three groups: (1) behavioral sleep-deprivation for 36 hours; (2) sleep-induced with sodium oxybate; (3) normal sleep (control). Sodium oxybate was selected for sleep-induction because it enhances slow wave sleep (SWS) (12). Each participant was invited to repeat the study and undergo randomization to a different group. Four participants repeated the study once and four participants completed all three groups. All participants were in good general health, had no clinical sleep or neurological diseases, and had no contraindication to a lumbar catheter. All participants

were screened to exclude sleep-disordered breathing with a home sleep apnea test (Apnealink, ResMed, San Diego, CA).

Polysomnography was performed as previously reported (5) throughout each participant's admission to the Clinical Research Unit. After an acclimation night, an intrathecal lumbar catheter was placed and collection of samples started in all participants at 07:00. All participants were kept awake from the time of lumbar catheter placement (07:00) until the start of the intervention (21:00). At 21:00, control participants were permitted to sleep and participants in the sleep-induced group received their first dose of sodium oxybate. A second dose of sodium oxybate was administered at 01:00. Sodium oxybate doses ranged from 3.25 grams to 3.75 grams at both 21:00 and 01:00 (total dose 6.5–7.5 grams/night) and were adjusted for each participant for a range of 45–55 mg/kg. Participants in the sleep-deprived group were kept awake by nursing staff and did not receive stimulants. The lumbar catheter was removed on day 2 at 19:00 and participants lay flat for 12 hours. Participants had meals served at 09:00, 13:00, and 18:00. Headaches were the most common adverse event and two participants required blood patches for spinal headaches (Table 1).

Sample Collection and A β SILK Analysis

Six mL of CSF were obtained every 2 hours for 36 hours. All samples were processed and measured as previously described (13). The procedure for stable isotope amino acid tracer administration, sample collection, and A β SILK tracer protocol using a 9-hour infusion of [U- $^{13}\text{C}_6$] leucine was performed as previously reported (13). A β SILK modeling was based on previously published models (13–15) with modifications necessitated by the reduced post-tracer sampling time compared to previous studies (22 vs. 36 hours). The model consisted of a plasma leucine precursor pool that generated labeled A β 38, A β 40, and A β 42 peptides. Each arm of the model consisted of a time delay process and a single compartment process; the length of the time delay and the single compartment fractional turnover rate (FTR) were independently adjustable for each peptide. The FTR represents the sum of irreversible losses of brain peptides to all processes (e.g. recovery in CSF, clearance through the blood brain barrier, and in situ proteolysis, uptake, or deposition). The production rate of each peptide was determined as the flux rate through the terminal CSF sampling site of the model. Compartmental modeling was performed using SAAM II (v. 2.3.1, The Epsilon Group, Charlottesville, VA). None of our participants had evidence of brain A β deposition based on A β 42:A β 40 >0.12, therefore exchange of A β 42 with an 'insoluble' compartment was not included. (13)

Statistics

Statistical analyses were performed using SPSS version 23 (IBM, Armonk, NY). All serial CSF A β data were analyzed with general linear mixed models in order to account for the dependencies among the longitudinal measurements (16). Intervention group and time of day were treated as fixed effects. Random intercepts and slopes for time were used to accommodate individual variation. The Akaike Information Criterion was used to compare covariance structures and compound symmetry was selected as the best fit. Statistical significance was set at $p < 0.05$. Bonferroni correction was used when making comparison between the three intervention groups (0.05/3). The normality assumption was verified

through residual plots. Differences in participant characteristics were also assessed with mixed models, as with the A β models, but without a time factor.

Results

A β concentrations were normalized to a baseline for each subject (average of hours 07:00–19:00) before each group's intervention. Mean overnight CSF A β 38, A β 40, and A β 42 concentrations increased 30% above baseline levels in sleep-deprived participants compared to the control and drug groups (Figure 1A–C; Table 2). There were no statistically significant differences between the control and drug groups. Figure 2 shows the change from baseline of A β 40 for each participant. A β 38 and A β 42 showed similar within-subject changes from baseline (data not shown). The time courses curves for $^{13}\text{C}_6$ -leucine isotopic enrichment were overlapping for A β 38, A β 40, and A β 42 (Figure 1D–F); the FTR of A β peptides is extremely sensitive to the shape of these curves (14). Ratios of labeled A β 38/40 and A β 42/40 were flat across the sampling period for all groups (Figure 1G–H), showing that the peptides had equivalent FTRs. Formal modeling analysis revealed that the FTRs and delay times were not significantly different between groups (Figure 1I; Table 2), which strongly suggests a lack of sleep pattern on the overall clearance processes of brain peptides. Since production rate is directly proportional to concentration in the model (14), overnight A β production rate in the sleep-deprived group was increased 30%.

Discussion

Diurnal oscillation of A β is hypothesized to result from 1) changes in A β production by neuronal activity (17, 18) and 2) changes in A β clearance via ISF bulk flow to cervical lymphatics and CSF during sleep (i.e. “glymphatic” drainage) (19). During wakefulness, A β concentration increases due to increased neuronal activity (production) and decreased ISF flow (clearance). Conversely, neuronal activity decreases and ISF flow increases during sleep that together account for a decrease in A β concentration. As predicted and consistent with prior work (8, 9), disrupted sleep resulted in higher overnight CSF A β levels in our study. The effect of disrupted sleep on overnight CSF A β levels in individual participants was variable, however (Figure 2). We hypothesize that this variability was due to factors not identifiable in our pilot study (age, gender, race, Apoe genotype).

The absence of group differences from the A β SILK puts constraints on the possible mechanisms that could account for the altered concentration time courses. We have previously shown that the shape of the kinetic curve is most sensitive to changes in clearance rates and is entirely unaffected by changes in production rate (14). We performed sensitivity analyses using example data from one participant and held all model parameters constant except for either production rate or FTR. When the production rate was 1% or 199% of nominal, the SILK curves were identical (Figure 3A). However, changes in FTR as small as 5% resulted in a noticeable change in peak shape and a small shift in peak time; 20% changes in FTR led to large changes in peak shape and peak time with separation of the labeling curves (Figure 3B–C).

Increased amyloid precursor protein (APP) production or increased cleavage of APP by β -secretase would increase the concentrations of all A β isoforms without altering the shapes of the SILK curves. In PSEN1 mutation carriers without amyloid deposition, for example, increased A β production does not alter the shape of $^{13}\text{C}_6$ -leucine labeling curves (14, 15). Further, soluble CSF APP metabolites fluctuate with a diurnal pattern (20) providing additional support that APP production is altered by sleep-wake activity. Sleep also did not alter labeled A β 38/A β 40 and A β 42/A β 40 ratios suggesting sleep does not affect β -secretase activity.

A change in overall clearance will affect the FTR of brain A β peptides, which is the sum of losses to CSF and all other fates. In order for decreased glymphatic clearance during sleep deprivation to increase soluble CSF A β , a decrease in irreversible losses (e.g. to the bloodstream or lymphatics) due to prolonged overnight waking would have to be perfectly matched by an increase in A β clearance to CSF. This is plausible but unlikely and not identifiable from the current data. The SILK kinetics results unequivocally show that glymphatic clearance alone, without compensation from other clearance mechanisms, would be ineffective in protecting the brain from AD because overall clearance rates are not changing. Glymphatic clearance may potentially contribute to the protective effects of sleep against AD, but changes in production rate seem to be the necessary and critical factor.

This pilot study provides the first evidence in humans that A β production is the mechanism for sleep-mediated changes in A β concentration. We did not find that increased SWS from sodium oxybate decreased A β compared to controls, possibly because time in SWS was not significantly different between the sleep-induced and control groups. Also, our study excluded participants with sleep disturbances; further decreasing A β with sleep enhancement may not be feasible in normal subjects. Given that there are many approved therapies targeting sleep, the effect of sleep-inducing drugs on CSF A β should be tested in individuals with sleep disruption and promising candidates investigated in AD prevention trials.

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Abbreviations

A β	amyloid- β
AD	Alzheimer's disease

APP	amyloid precursor protein
CSF	cerebrospinal fluid
FTR	fractional turnover rate
ISF	interstitial fluid
MMSE	Mini-Mental Status Examination
SILK	stable isotope labeling kinetics
SWS	slow wave sleep

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Figure 1. Overnight CSF Aβ kinetics

Eight subjects participated in the study. Four participants completed the control, the sleep-deprived, and sleep-induced intervention groups. Four participants completed two groups. A–C: All Aβ38, Aβ40, and Aβ42 concentrations were normalized to the baseline 07:00–19:00. The overnight period during the intervention night was defined as hours 18–28 (01:00–11:00) to account for transit time of CSF from the brain to lumbar catheter (shaded). Sleep deprivation increased overnight Aβ by 30% over the baseline compared to participants in the control and sleep-induced groups, however the control and sleep-induced groups were not significantly different. D–F: Aβ peptide enrichments were normalized to each subject's plasma leucine enrichment plateau. The shape of the normalized SILK curves were similar between groups for Aβ38, Aβ40, and Aβ42. G–H: Aβ38/40 and Aβ42/40 isotopic enrichment ratios from hours 18–36 (01:00–19:00). I: Fractional turnover rates (FTR) for Aβ38, Aβ40, and Aβ42 were not significantly different between groups. Blue=control; Red=sleep-deprived; Green=sleep-induced. Error bars indicate standard error. The vertical dashed line is both the time of ¹³C₆-leucine infusion and the intervention start time. The horizontal dashed line is at 100% of baseline. *p<0.0001.

A β 40

Figure 2. Within-subject changes in A β 40 from baseline

4 participants completed the control (blue), sleep-deprived (red), and sleep-induced (green) intervention groups (A–D). 4 participants completed two groups (E–H). All A β 40 concentrations were normalized to the baseline 07:00–19:00. The overnight period during the intervention night was defined as hours 18–28 (01:00–11:00) to account for transit time of CSF from the brain to lumbar catheter (shaded). The vertical dashed line is both the time of $^{13}\text{C}_6$ -leucine infusion and the intervention start time. The horizontal dashed line is at 100% of baseline.

Figure 3.

Aβ SILK model sensitivity analyses. Stable isotope labeling kinetic (SILK) modeling of normalized mole fraction labeled of Aβ40 from one participant was performed (black). A. All model parameters were held constant except for production rate. Production rate was changed to 199% (green) and 1% of the nominal production rate (red). Production rate did not alter the best model fit of the SILK data and the labeling curves are identical. B. All model parameters were held constant except for fractional turnover rate (FTR). FTR was changed to 105% (green) and 95% (red) of the nominal FTR. Increased FTR by 5% results in a higher and earlier peak (green dashed line) compared to decreasing FTR by 5% (red dashed line). C. All model parameters were held constant except for fractional turnover rate (FTR). FTR was changed to 120% (green) and 80% (red) of the nominal FTR. Increased FTR results in a higher, narrower, and earlier peak (green dashed line) while decreased FTR delayed peak onset and widened the labeling curve (red dashed line).

Table 1

Participant Characteristics

		Control (N=7)	Sleep-deprived (N=7)	Sleep-induced (N=6)
Age (years)	Mean	47.6	48.1	51
	SD	9.2	10	9.8
Sex (Male/Female)		2/5	3/4	3/3
Race (C/AA)		3/4	3/4	2/4
Body mass index (kg/m ²)	Mean	26.9	27.1	26.6
	SD	4.3	3.6	3.3
Mini-Mental Status Exam	Mean	28.9	28.8	28.6
	SD	0.7	0.8	0.5
Aβ42:Aβ40	Mean	0.188	0.186	0.163
	SD	0.07	0.03	0.04
Total Sleep Time (min)	Mean	403.5	22.8[*]	432.3
	SD	54.4	24.5	50.9
Sleep Efficiency (%)	Mean	75.7	3.7[*]	78.0
	SD	8.9	4.7	7.7
Time in Slow Wave Sleep (min)	Mean	14.2	0	69.3[#]
	SD	20.1	0	74.8
Sodium oxybate (mg/kg)	Mean			47.56
	SD			4.91
Adverse Events				
<i>Headaches</i>		5/7	2/7	4/7
<i>Blood patch</i>		0	1	1
<i>Nausea, vomiting</i>		0	1	0
<i>Leg tingling</i>		0	1	0
<i>Back/neck pain</i>		1	0	0

Intervention groups include repeat participants and are not independent groups. Data is shown this way to highlight effect of sleep conditions in each group. Significance tests for treatment differences were made using mixed models to accommodate the non-independence of the measurements.

C: Caucasian; AA: African-American; kg: kilograms; m: meters; Aβ: amyloid-β; min: minutes; mg: milligrams; SD: standard deviation

^{*} Total sleep time and sleep efficiency were significantly lower in the sleep-deprived condition compared to the control and sleep-induced condition (Total sleep time: F(2,13)=159.3, p<0.0001; Sleep efficiency: F(2,12)=213.8, p<0.0001).

[#] Sleep-induced condition differed significantly from the sleep-deprived condition (Time in Slow Wave Sleep: F(2,14)=4.51, p=0.03).

Table 2

Mixed Model Results for Overnight A β and A β Stable Isotope Labeling Kinetics

Overnight A β (0100–1100)					
A β	Factor	Pairwise Comparison	Mean Difference	F (df)	p-value
A β 38 (% baseline)	Intervention			18.51 (2,98)	<0.0001
		SD vs. Control	+28.5%		<0.0001 *
		SD vs. SI	+30.9%		<0.0001 *
		Control vs. SI	+2.4%		1.0 *
	Time of Day			0.557 (5,94)	0.73
A β 40 (% baseline)	Intervention x Time			0.568 (10,94)	0.836
	Intervention			18.73 (2,98)	<0.0001
		SD vs. Control	+27.9%		<0.0001 *
		SD vs. SI	+25.8%		<0.0001 *
A β 42 (% baseline)		Control vs. SI	–2.1%		1.0 *
	Time of Day			1.14 (5,94)	0.346
	Intervention x Time			0.399 (10,94)	0.944
	Intervention			16.95 (2,98)	<0.0001
		SD vs. Control	+29.1%		<0.0001 *
		SD vs. SI	+29.9%		<0.0001 *
		Control vs. SI	+0.8%		1.0 *
	Time of Day			0.74 (5,94)	0.596
	Intervention x Time			0.444 (10,94)	0.921
A β Stable Isotope Labeling Kinetics					
FTR A β 38 (pools/hr)	Intervention			1.117 (2,12)	0.36
		SD vs. Control	–0.023		1.0 *

Overnight A β (0100–1100)					
A β	Factor	Pairwise Comparison	Mean Difference	F (df)	p-value
		SD vs. SI	–0.056		0.484 *
		Control vs. SI	–0.033		1.0 *
FTR A β 40 (pools/hr)	Intervention			1.078 (2,12)	0.372
		SD vs. Control	–0.008		1.0 *
		SD vs. SI	–0.04		0.551 *
		Control vs. SI	–0.032		0.901 *
FTR A β 42 (pools/hr)	Intervention			1.921 (2,11)	0.191
		SD vs. Control	0.001		1.0 *
		SD vs. SI	–0.05		0.328 *
		Control vs. SI	–0.051		0.348 *
A β 38 Delay Time (hr)	Intervention			1.835 (2,12)	0.202
		SD vs. Control	–1.211		0.364 *
		SD vs. SI	–1.152		0.39 *
		Control vs. SI	0.06		1.0 *
A β 40 Delay Time (hr)	Intervention			1.458 (2,12)	0.271
		SD vs. Control	–0.962		0.464 *
		SD vs. SI	–0.871		0.56 *
		Control vs. SI	0.091		1.0 *
A β 42 Delay Time (hr)	Intervention			1.54 (2,12)	0.253
		SD vs. Control	–0.864		0.512 *
		SD vs. SI	–0.904		0.429 *
		Control vs. SI	–0.041		1.0 *

Significance tests for group differences were made using mixed models.

A β : Amyloid- β ; SD: Sleep-deprived; SI: Sleep-induced; df: degrees of freedom; FTR: Fractional Turnover Rate; hr: Hour

* Bonferroni corrected for multiple comparisons