

Review

Alzheimer's Disease Can Be Spared by Nonsteroidal Anti-Inflammatory Drugs

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Abstract. Alzheimer's disease (AD) is characterized by deposits of amyloid- β protein ($A\beta$) in brain which become foci of inflammation. Neurons are destroyed by this inflammatory process, leading to the cognitive deficits which define AD clinical onset. Epidemiological studies indicate that nonsteroidal anti-inflammatory drugs (NSAIDs) can ameliorate this destructive process if they are started well before clinical signs develop. Biomarker studies indicate that the disease process starts at least a decade before cognitive deficits appear. This pre-clinical onset explains the NSAID effect. It also opens a window of opportunity for preventive treatment that can be met with a simple diagnostic test. Salivary levels of $A\beta_{42}$ may fulfill that need. They can be measured by a simple ELISA test we have developed using commercially available reagents. By this ELISA test, normal controls, who are not at risk for AD, have levels of $A\beta_{42}$ close to 20 pg/ml. AD cases, as well as high level controls, secrete levels in the range of 40–85 pg/ml. Widespread application of this test to detect high level controls, followed by NSAID consumption, could substantially reduce the prevalence of AD.

Keywords: $A\beta_{42}$, epidemiology, neuroinflammation, pre-clinical Alzheimer's disease, saliva

HISTORICAL BACKGROUND

The modern era of brain research was initiated by investigations into the causes of Alzheimer's disease (AD). We reported in 1988 that AD brains showed activated microglia expressing HLA-DR, an immunology marker previously thought to be exclusively associated with peripheral leukocytes [1]. The myth of brain immunological privilege was thus demolished. It did not go down without a fight. Our first attempt to publish on the subject was rejected by the editor of one prominent journal who was certain the result must be wrong, but was unable to put a finger as to why. The editor decided that any neuropathologist could do so and rejected the paper on the basis of our not having a neuropathologist as an author. Our grant application to support the research

was rejected when a peer reviewer evaluated it as a "crazy hypothesis".

As with all breakthroughs, it caused a paradigm shift in thinking. Once that shift took hold, progress became rapid. The result is that today neuroinflammation is the largest field of brain research. Departments of neuroinflammation have now become established in academic institutions to meet the demand.

Epidemiological data soon backed up our immunohistochemical results. In this field, there was less controversy since new methodology was not involved. In 1990, we wrote a short report for *Lancet* [2] indicating a substantial sparing of AD in patients with rheumatoid arthritis. We suggested that anti-inflammatory therapy might be the explanation. We chose rheumatoid arthritis for the study since it typically commences at an earlier age than AD, and is universally treated with anti-inflammatory agents.

Our report of AD sparing in patients consuming anti-inflammatory agents was soon confirmed

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in 17 epidemiological studies [3]. They included incident, prevalent, and case-control studies of patients consuming nonsteroidal anti-inflammatory drugs (NSAIDs) compared with controls. There was one consistent caveat in these epidemiological studies. The NSAIDs needed to have been started at least 6 months, and preferably as long as 5 years, before the clinical diagnosis of AD.

DEVELOPMENTAL YEARS

A new field of research had been opened up with these epidemiological studies. It required that some important questions be answered. Why was it necessary to commence taking NSAIDs so long before the clinical onset of AD? What was the appropriate NSAID dose? And was it necessary to take NSAIDs on a continuing basis?

New techniques were required to provide answers to these questions. We emphasize here the two most important of these: positron emission tomography revealing that deposits of amyloid- β protein ($A\beta$) build up in the brain of AD cases; and cerebrospinal fluid (CSF) $A\beta$ levels revealing their consequent reduction. These two techniques are complementary. Since the brain $A\beta$ deposits accumulate over time, the effect is integral. Since CSF turns over every few hours, the effect is differential.

Disease development, as revealed by biomarker studies, follows this sequence of events. It commences with $A\beta$ deposits developing in the brain of AD cases. These deposits can be detected by positron emission tomography (PET) using the $A\beta$ binding agent Pittsburgh Compound B. The depositions result in a concomitant decrease of $A\beta$ in the CSF. Years later, less definitive biomarkers such as MRI and regional metabolic rate by fluorodeoxyglucose (FDG)-PET become positive. These later biomarkers reveal loss of brain tissue. When they become positive, cognitive deficits have already appeared. Together, these studies indicate that AD onset commences more than a decade before clinical signs develop [4–7]. The ability to identify the onset of AD a decade or more before clinical signs appear creates a window of opportunity to intervene in the process. Moreover, it explains the epidemiological data in which NSAIDs must be commenced years before clinical detection.

The missing link is a simple, non-invasive method for identifying those at risk at an age well below the typical age of AD onset.

PROVIDING THE MISSING LINK

Analysis of saliva for $A\beta_{42}$ may provide the missing link. However, the data we present is preliminary and will need to be confirmed by further studies. We first developed a simple ELISA method for determining $A\beta_{42}$ levels in tissues as well as saliva [8]. The results showed that $A\beta_{42}$ is produced in all tissues of the body, and not just in brain as many have believed.

$A\beta_{42}$ secretion in saliva is a reflection of its production by submandibular glands. The results are shown in Table 2 for a series of AD and non-AD cases. The non-AD cases resolved into two distinct categories: those with low levels in the 19–25 pg/ml range, and those with high levels in the AD range of 41–60 pg/ml. Significantly, there were no overlapping cases.

Analysis of $A\beta_{42}$ levels in saliva demonstrates three remarkable facts. Firstly, controls, who are not at risk for AD, secrete levels close to 20 pg/ml, regardless of sex or age (see Table 1). Secondly, this production is constant, being invariant with time of day, and from day to day. Thirdly as Table 2 shows, those at risk for AD secrete levels comparable to AD cases [8].

The demonstration of a constant activity of genes, and their expression everywhere in the body, requires a paradigm shift in our concept of how many genes function. They are expressed in every tissue at a constant rate around the clock, with no variation throughout life. We are currently investigating whether NSAID consumption can affect these levels.

A theoretical construct suggests the development of AD goes through 6 phases, each with decreasing opportunity for therapeutic intervention [9]. Since the prevalence of clinical AD commences at age 65, the prevalence for actual AD disease onset can be hypothesized to occur at least 10 years earlier, or at age 55. Without intervention, the prevalence will then

Table 1
 $A\beta_{42}$ levels in human organs measured by our ELISA method

Human tissue	$A\beta_{42}$ levels (pg/ml)
Lung	83.68
Heart	88.59
Kidney	122.65
Hippocampus	102.45
Sensory Cortex	97.83
Liver	90.56
Spleen	134.38
Small intestine	80.58
Pancreas	128.22

Table 2
A β ₄₂ levels in normal and AD cases measured by our ELISA method

Normal (Low controls)				AD				Normal (High Controls)			
Case	Age	Gender	A β ₄₂ (pg/ml)	Case	Age	Gender	A β ₄₂ (pg/ml)	Case	Age	Gender	A β ₄₂ (pg/ml)
1	46	Male	25.32	1	52	Female	59.57	1	71	Female	48.31
2	53	Male	24.44	2	60	Female	47.96	2	79	Male	45.28
3	89	Male	22.35	3	57	Male	41.58	3	52	Female	59.57
4	16	Male	21.47	4	72	Female	41.69	4	70	Female	41.12
5	19	Male	20.27	5	77	Female	84.97	5	71	Male	39.66
6	19	Female	19.73	6	78	Male	48.89	6	71	Male	41.83
7	22	Male	22.27	7	84	Female	64.93				
8	24	Male	21.53	8	86	Female	75.20				
9	39	Male	22.22	9	91	Male	56.67				
10	42	Male	20.58	10	84	Male	49.94				
11	47	Female	20.15	11	78	Female	55.93				
12	54	Male	20.05	12	75	Female	47.93				
13	54	Female	20.56	13	75	Female	60.68				
14	55	Female	29.66	14	74	Male	63.73				
15	54	Male	22.32	15	8	Male	42.77				
16	57	Female	19.61	16	72	Female	45.95				
17	57	Female	23.07	17	64	Female	53.83				
18	59	Male	21.55	18	48	Female	49.26				
19	60	Male	22.02	19	78	Female	58.28				
20	62	Male	21.37	20	83	Male	44.19				
21	78	Male	21.44	21	80	Female	51.11				
22	83	Female	20.56	22	79	Male	49.07				
23	86	Male	21.37	23	84	Female	46.62				
24	88	Male	22.46								
25	92	Female	20.89								

Values are Mean \pm SEMs; Normal (Low controls): 21.54 \pm 0.19, AD: 53.95 \pm 2.24, and Normal (High controls): 45.96 \pm 3.01, respectively. One-way ANOVA was carried out to test significance. $p < 0.001$ for AD and Normal (High controls) cases compared with Normal (Low controls).

double every five years. It is characterized by the start of A β deposition in brain with consequent decreases in the CSF. Therapeutic opportunities at this initial stage are at their highest. Any strategy which limits A β production, enhances its clearance, or prevents its aggregation, should be disease modifying. Effectiveness of treatment should be measurable by CSF A β levels returning toward normal.

Biomarker studies suggest that phase 2 sets in about 5 years later. A β deposits in the brain have built up to the level where they can be easily detected by PET scanning with Pittsburgh compound B. A β decreases in the CSF continue. They are now joined by tau levels. Therapeutic opportunities have declined because cortical tau aggregation in brain has been induced. Ideally, a tau aggregation inhibitor should now be added to any therapeutic regimen, but so far none have been produced. Given that tau aggregation is the central pathogenic event in more than two dozen neurodegenerative diseases, developing such an agent is an urgent priority.

In phase 3, typically after another 5 years, there is a slight metabolic decline that can be demonstrated by a reduction in FDG uptake by PET scanning.

Presumably this is due to synaptic loss. Cortical tangle and thread development have occurred. A β and tau continue to be expressed at the same levels in the CSF. Pittsburgh compound B scanning intensity has increased. Therapeutic opportunities have further declined.

Phase 4, in another 5 years, represents disease progression to the level where mild cognitive impairment is detectable. Hippocampal atrophy becomes evident by MRI scanning. The disease can no longer be totally prevented. Irreversible brain damage has begun to occur. A β and tau continue to be expressed at the same levels in the CSF.

Phase 5, in another 5 years, represents the level where AD can easily be diagnosed clinically. Neuronal damage and loss is evidenced by further PET-FDG metabolic decline and increasing brain volume loss by MRI. A β deposits in brain continue to accumulate. Opportunities for therapeutic intervention have seriously declined.

Phase 6 defines the period of progressive clinical decline in all aspects of the disease. Cognitive deficits progress from mild to severe. Full-time care of patients becomes necessary. Costs associated with

their care escalate. Therapeutic opportunities are minimal and yet it is in this phase that most clinical trials have been conducted. The result has been a failure in 242 of the last 243 AD clinical trials. The one exception was for memantine, a me-too agent affecting acetylcholine production rather than AD itself. The situation fits Albert Einstein's definition of investigator insanity. That is to do the same experiment over and over hoping for a different answer.

To be a truly successful disease-modifying agent, a therapeutic candidate needs to deter A β ₄₂ aggregation, or intervene in its consequences, prior to the time when tau aggregation takes hold. For example, early application of direct inhibitors of A β aggregation, or inhibitors of β -secretase, or γ -secretase might succeed. Active immunization is highly questionable, given the dangers of inducing an autoimmune disorder and stimulating self-attack by complement. Passive immunization is also unlikely to succeed, due to the problems of blood-brain barrier penetration. Repeated failures of such agents have already been demonstrated.

DISCLOSURE STATEMENT

Authors' disclosures available online (<http://j-alz.com/manuscript-disclosures/17-0706r1>).

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