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Lost in Translation? Finding Our Way To Effective Alzheimer's Disease Therapies

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Abstract

Efforts over the past two decades to develop effective disease-modifying treatments for Alzheimer's disease have been disappointing, while parallel efforts in another chronic neurologic disease, multiple sclerosis, have been remarkably productive. In an effort to advance development of therapeutics for Alzheimer's disease, these two fields are contrasted in terms of the utility of animal models, definition of study populations, and utility of biomarkers. Possible solutions are suggested, and the review concludes with description of some active peer-reviewed, publicly funded clinical studies which address some of the identified weaknesses in past clinical trials for age-related dementia.

Keywords

Alzheimer's disease; animal models; clinical trials

INTRODUCTION

The inability to demonstrate disease-modifying effects in Alzheimer's disease (AD) has been a great frustration for patients, clinicians, and investigators. These repeated failures may be due to over-reliance on invalid hypotheses, an unfortunate choice of specific interventions, problems with clinical trial design, or myriad other explanations. Debates on these points are rarely conclusive, in part because there is not a positive outcome to contrast with all of the negative results and make the case for paradigm change in hypothesis or in trial design. Since failure is the norm in AD therapeutics research, we will need to look to other fields to find examples of success to guide us. We will consequently proceed with a brief review of a representative failed clinical trial in AD, and then consider the elements of success in a more productive field (multiple sclerosis therapeutics). We will then conclude with some thoughts about where the field should move in the future.

A REPRESENTATIVE CLINICAL TRIAL: THE NIA-ADCS DHA TRIAL TO SLOW THE PROGRESSION OF AD [1]

The decision to conduct a multi-center trial of an omega 3 fatty acid for AD, at a cost of approximately ten million NIH dollars, was the result of a long, thoughtful, systematic deliberation by the Alzheimer's Disease Cooperative Study (ADCS) leadership and Steering Committee. The process started with submission of a protocol synopsis to the Project

Selection Committee, which selected the study for presentation at a meeting of the Steering Committee, comprised of approximately 35 AD clinical trial experts from the various sites. After the Steering Committee selected the omega 3 trial for further development, the protocol was then presented to an independent Scientific Advisory Board. After the scientific advisors also endorsed the protocol for further development, the project was included as one of five clinical trials in the competitive renewal application for the ADCS and went to NIH study section. The study section recommended funding the trial. In other words, this trial was initiated only after unusually thorough peer review, so retrospective consideration of the final outcome cannot point to lack of due diligence in explaining the failure to find therapeutic benefit with this strategy. The rationale for the study was two-fold: 1) abundant epidemiologic data indicated that dietary consumption of fish and/or omega 3 fatty acids was associated with a lower incidence of dementia [2] and 2) studies in transgenic mouse models of AD demonstrated that supplementation of the omega 3 fatty acid docosahexaenoic acid (DHA) produced anti-amyloid and neuroprotectant effects [3, 4]. The animal studies chose DHA as the specific omega 3 fatty acid for supplementation because DHA is the most abundant polyunsaturated fatty acid in the brain and is enriched in synaptic fractions, whereas eicosapentaenoic acid (EPA), the other omega 3 fatty acid thought to be responsible for the health benefits of fish consumption, is found at only very low concentrations in brain tissue.

The DHA trial was enrolled on time, compliance was excellent, DHA was well tolerated, and retention was within the anticipated range [1]. Unfortunately, however, the DHA-treated participants progressed at the same rate as the placebo-treated participants, and this negative result was reported in 2010 [1]. A pre-specified subgroup analysis showed a statistically significant effect of DHA treatment upon one of the two primary outcome measures in the ApoE4 non-carriers, which comprised about half of the overall study population. However, there was no effect of DHA treatment in the non-carriers on the other co-primary outcome, nor on any of the secondary outcome measures, so the isolated positive finding with one outcome measure in E4 non-carriers was reported but not emphasized in the final publication [1].

Reasons for failure

In retrospect, some of the possible reasons for the failure of this trial to find a therapeutic benefit of DHA include:

- 1) Dependence on animal models that are not predictive of clinical outcomes.**—The APP mice used in the preclinical studies [3, 4] have been used to screen hundreds of candidate therapies, many of which have moved on to clinical trials, and none of which have been shown to have robust clinical effects.
- 2) Insufficient attention to the study population.**—Epidemiologic data pre-dating the trial suggested that the brain health benefits of omega 3 fatty acids may be more evident in E4 non-carriers compared to carriers [5, 6], so confining a trial to non-carriers may have yielded a different result. Subsequent investigations have explored the possibility that E4 carriers may be unable to benefit from DHA because of genotype-associated impairment in

DHA uptake [7]. Natural history studies and other clinical trials also suggest that any intervention targeting amyloid- ($A\beta$) may need to be initiated early in the disease course, before the onset of overt dementia, in order to achieve a therapeutic benefit, so the mild to moderate AD population targeted in this trial may have been too far along in the disease course to benefit from any anti- amyloid intervention. In fact, a *post hoc* analysis of another omega 3 fatty acid trial in AD suggested the benefit was confined to the most mildly affected individuals [8], so a focus on early AD or MCI might have increased the chance of seeing a therapeutic effect.

3) Sacrificing the rationale for the study to the practicalities of clinical trial design.—The rationale for the DHA study was the observation that omega 3 fatty acid intake was associated with a lower risk of AD, but the clinical trial evaluated the effects of omega 3 on disease progression, rather than on disease onset. This decision was a practical concession to the fact that prevention studies require thousands of subjects followed for several years, while a treatment trial can be powered to detect an effect with hundreds of subjects followed for 18 months. However, we have several lines of evidence to suggest that mechanisms of disease underlying AD initiation and AD progression are not identical. For example, ApoE genotype seems to be important for disease initiation but not necessarily for disease progression. The ADNI [9] and DIAN [10] biomarker data illustrate that different phases of AD initiation and progression are marked by shifts in different biomarkers, suggesting that the mechanisms of disease also change over the course of the disease. This extrapolation from evidence for *prevention* effects to testing of *treatment* effects has also been applied in other trials of NSAIDs [11], statins [12], and homocysteine-lowering vitamins [13] for AD, with similar negative outcomes in each of those trials. Other transitions from epidemiologic observation to trial design may also exert confounding effects. For example, the use of pure DHA rather than mixed omega 3 fatty acids in the DHA trial did not directly follow from the epidemiology, which evaluated consumption of omega 3 fatty acids as they exist in the diet, with DHA and EPA in combination. Since EPA may have greater effects than DHA on some end-points like vascular health, the decision to focus on a single omega 3 fatty acid may have compromised the ability to find a treatment effect.

4) Absence of surrogate outcome measures for a “proof of concept” clinical trial as an intermediate step between transgenic mouse studies and full-scale clinical trial.—This practice of leaping from animal studies to full-scale trials has been repeated many times in the effort to develop AD therapeutics and will likely continue until a paradigm for evaluating candidate therapies in smaller cohorts is shown to predict effects in full-scale trials. The protocol for serial sampling of radio-labelled cerebrospinal fluid (CSF) $A\beta$ via lumbar drain in human subjects is a promising example of an informative “proof of concept” design [14] which may permit rational “go-no go” decisions [15] in the evaluation of experimental agents for AD, but this method is technically demanding and there is ample room for additional outcome measures for this purpose.

A REPRESENTATIVE SUCCESS STORY: THERAPY DEVELOPMENT IN MULTIPLE SCLEROSIS

The repeated successes in multiple sclerosis (MS) over the last 20 years stand in sharp contrast to the repeated failures in AD drug development during the same time frame. It may be instructive to attend to elements that may have promoted success in the MS field, contrasting them with the four points listed above:

1) Identification of an animal model predictive of clinical effects.

Many (although not all) disease-modifying drugs for MS were initially evaluated in the experimental allergic encephalomyelitis (EAE) mouse model of MS. While the EAE model is by no means a perfect model of the human disease, it is clearly a useful model for drug development [16]. The arguments about the relative value of different animal models in the AD field, including the argument that no AD animal models are valid, miss the point illustrated by EAE and the development of MS drugs: animal models need not be perfect in order to predict clinical outcomes.

2) Careful attention to definition of study population.

The landmark trial of beta-interferon, the first disease-modifying drug approved for MS, was at the time novel in its restriction of the study population to patients with a particular phenotype (relapsing- remitting) and an established level of disease activity (two relapses within the last year) [17]. Once proven in the beta-interferon study, this aspect of trial design became standard in MS drug development, leading to the approval of 15 disease-modifying drugs for relapsing-remitting MS at last count.

3) Rational extrapolation from preclinical data to clinical trial design.

Since the EAE model is a model of the inflammatory aspect of MS, trials based on this model have targeted the clinical correlate: new lesions and clinical relapses in MS. While it is increasingly recognized that some of the chronic progressive features of MS are non-inflammatory, the field was not hindered by an effort to treat all aspects of the disease in each clinical trial.

4) Validation of a surrogate endpoint predictive of clinical outcome.

The original beta-interferon trial was also novel in the inclusion of MRI lesion count as an outcome measure [18]. This was innovative at that time, but once its utility was demonstrated, the reliance on surrogate outcome measures has become standard, routine, and beyond question. Similar surrogate outcome measures will be necessary to develop a paradigm for a “proof of concept” trial design in AD.

LOOKING TOWARDS A MORE SUCCESSFUL FUTURE IN AD DRUG DEVELOPMENT

A “wish-list” for future directions in AD therapeutic development may be organized along the same four points listed above in reasons for success and failure:

1) Rational use of animal models.

There may be occasional opportunities for launching clinical trials without animal data, but we are likely to continue to use animal models as a prelude to clinical trials in AD, despite their imperfections. Frustrations with animal models in other neuroscience arenas have given rise to the “STAIR” recommendations for pre-clinical evaluation of stroke therapies [19], and to the Michael J. Fox Foundation’s current requirement that efficacy of any candidate therapy must be demonstrated in two distinct animal models before moving forward to clinical testing. However, neither of these strategies has yet borne fruit, clinically speaking, so it remains to be seen whether these types of recommendations are the best path forward. Therapeutic development in AD is unlikely to be advanced by generation of more animal models or by continued debate over the relative merits of specific models or of animal models in general, keeping in mind the mantra, illustrated in the EAE-MS example, that “All models are bad, but some are useful.”

Instead of revising our preclinical models, we may render animal models more useful by more careful attention to early clinical trial design. Early clinical trials should define a study population, disease stage, and dose and duration of treatment that follow logically from animal studies. Greater efforts to include “translatable biomarkers” in preclinical studies will also facilitate effective translation to clinical trials.

2) Careful attention to study population in clinical trials.

The AD therapeutics field is attending to this issue in several important ways. There is general agreement that a clinical trial study population should be as homogeneous as possible; the challenge is finding the ideal subjects. For example, the ideal study population for clinical testing of drugs shown effective in transgenic mouse models of autosomal dominant AD would be human beings who express those genes, but the rarity of those mutations is limiting. Clinical trials in the DIAN cohort [20] and in a large Columbian kindred with autosomal dominant AD [21] are examples of how this idealized study population can be assembled, but the numbers of candidate therapies that can be evaluated with these extraordinary approaches remains quite limited.

While these carriers of well-defined highly penetrant genes for autosomal dominant AD are very rare, there may be a larger population of AD patients who, like these gene carriers, exhibit increased rates of A β ₄₂ synthesis, so may be more ideal candidates for therapies that attenuate A β production rates. Although the vast majority of late onset AD seem to have deficits in clearance, rather than synthesis of A β [22], patients with early onset AD who are not gene carriers may nevertheless have increased rates of A β production (based on the observations with the known genes for autosomal dominant AD). It may be possible to characterize these individuals with A β production rates in patient-derived fibroblasts or induced pluripotent stem cells in order to identify a cohort of A β over-producers for a targeted clinical trial.

Some trials are also defining study populations using more common genetic risk polymorphisms such as ApoE. The ideal trial in this population would be directed at the mechanism by which ApoE promotes AD, but that trial will require a more complete

understanding of ApoE pathophysiology than is currently available. In the meantime, E4 carrier status may be used to select for risk of decline in individuals with mild impairments and may also be applied in *post hoc* analyses divided by ApoE status to identify genotype-specific treatment effects (as suggested in ADCS-DHA trial).

Moving beyond genetic definition of the study population, the AD field has also moved toward a requirement for biomarker evidence of amyloid pathology as inclusion criteria for participation in anti-amyloid trials aiming at disease prevention or very early intervention, and this is expected to improve the chances of detecting treatment effects.

There are also some low-tech approaches that may be considered, starting with limiting study populations by age. For example, if older AD patients tend to have a greater degree of non-amyloid pathology underlying their clinical AD diagnosis compared to younger patients, then confining anti-amyloid trials to the younger patients who are more likely to have “pure” amyloid pathology will increase the likelihood of finding treatment effects. This raises the specter of “age-ism” and other concerns, but in light of increasing evidence that clinical AD in older patients is pathologically distinct from younger patients, matching the study population to the intervention is at least rational, and may even be essential for detecting therapeutic effects with these approaches.

Another “low tech” approach may be to require study participants to demonstrate a given level of disease activity in the year prior to study entry, along the lines of the requirements for MS trials. In the case of AD, this would mean evidence of a given rate of change over time, which would mean monitoring potential drug study candidates before randomization.

A final low-tech approach involves minimizing non-genetic heterogeneity, recognizing that environmental differences in study populations are a major confounder in the effort to identify treatment effects. This might best be achieved by efforts to promote healthy brain habits (e.g., vascular risk optimization, optimal nutrition, optimal sleep, exercise) among potential trial participants. While this would require considerable effort, imagine if the NIA-funded Alzheimer’s Centers were commissioned to create a “trial-ready” cohort of subjects rather than continue another 30 years of natural history studies. Subjects could be genotyped, biochemically pheno-typed (in terms of A β production rates, for example), coached and optimized in consensus best practices for optimal brain health, phenotyped in terms of rate of progression, and then delivered to target-specific clinical trials.

3) Clinical study designs that follow logically from the rationale.

Clinical trial design frequently deviates from the original rationale of the study for a variety of reasons. For example, in recognition of the fact that “ideal” subjects do not exist in sufficient numbers to fill a trial, inclusion-exclusion criteria are typically not as stringent as they should be at the time of protocol design, and they are invariably loosened further when recruitment falls behind schedule.

Further trial design compromises may be made for the sake of intellectual property. For example, in the ADCS-DHA study, part of the rationale for focusing on DHA rather than mixed omega 3 s arose from the opportunity for co-sponsorship of the study from

manufacturers of a proprietary form of “pure” DHA. As described above, this departure from the epidemiological data may have reduced the potential for seeing a therapeutic effect. We describe below a “second effort” with omega 3 s which is more faithful to the epidemiology and may have a greater chance of achieving success.

4) Development of surrogate outcome measures that predict clinical outcomes.

The ultimate validation of a surrogate outcome measure will depend on the demonstration of concomitant effects on the surrogate measure and on clinically important outcomes. In the MS world, this occurred with the original beta-interferon trial and set the course for MS drug development for the next 20-plus years. This may be achieved in AD with currently available CSF protein biomarkers, MRI measures, or PET measures, but the expense, duration of follow- up required, and insensitivity to short term change in brief proof-of-concept trials limit each of these modalities. The development of biomarkers which are more sensitive to change in the short term could greatly accelerate the pace of therapeutic development. The development of biomarkers which extend beyond A β and tau pathology may also facilitate development of effective interventions.

We describe below three examples of alternative surrogate outcome measures being developed in studies which are currently underway:

1. The NIA-funded trial “PUFAs for the prevention of vascular cognitive impairment” (coPI’s Shinto and Bowman) is an example of a single site trial of a therapeutic strategy which is strengthened by a rigorous definition of study population, an intervention that is true to the original rationale [23], and a surrogate outcome measure that reduces the numbers of subjects and duration of time needed to detect a treatment effect.

The hypothesis is that omega 3 fatty acid supplementation will reduce the progression of white matter hyperintensities in elderly subjects at risk of vascular cognitive decline, based on observations by us and others of a strong relationship between white matter hyperintensity burden and plasma omega 3 status [23]. The study population is defined by the absence of dementia, low baseline plasma levels of omega 3 s, and a requirement for a threshold level of white matter hyperintensity at baseline. The intervention is fish oil with EPA and DHA in the combination which drives the observational studies which motivated the trial, and which combines the potential neuroprotectant effects of DHA with the potential vascular benefits of EPA. The primary outcome measure is the rate of accumulation of white matter hyperintensities over three years. The trial is fully enrolled with 100 participants recruited at Oregon Health and Science University and clinical activity will be completed in 2019.

2. An NCCIH-funded clinical study of a botanical treatment for AD (PI Soumyanath) exemplifies the use of a relatively novel surrogate outcome measure for early clinical development of an AD treatment developed in an animal model. Studies in cell culture and animal models have demonstrated that *Centella asiatica* has potential as a therapeutic agent for AD because the extract attenuates mitochondrial dysfunction induced by A13 [24–28]. In order to

determine if these effects can be achieved in human subjects, a surrogate measure of brain energy production utilizing phosphorus NMR-spectroscopy will be employed in a preliminary dose-finding study. Phosphorus-NMR measurement of brain energy status has been employed productively in similar early phase clinical trials in Huntington's disease [29], but is relatively novel in AD and is well-suited to this trial based on the preclinical data. This study is at a very early stage, with IND application still under way.

3. An NCATS-funded study of CSF microRNA as biomarkers of AD (PI Saugstad) is another example of an effort to develop novel outcome measures for proof-of-concept trials in AD. Funded as part of the extracellular RNA consortium [1], this effort involves refinement of protocols for isolating and quantifying RNA from CSF [30], and initially generated 26 candidate biomarkers from a panel of 756 miRNAs [30]. Validation of these findings in an independent sample is under way at present, and future plans include evaluation of plasma miRNA, evaluation of specificity for AD, effects of disease stage, and others. The hope is that this miRNA panel will identify new target pathways and will also serve as a rapidly responsive read-out of therapeutic effects in brief proof-of-concept clinical trials. In conclusion, we have identified several key areas for improvement in the development of AD therapeutics, and we have initiated efforts to move the field forward. The repeated successes in other areas of clinical neuroscience prove that this is not futile, provided we learn from both the failures and successes of the past two decades.

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