Antibiotics in Dementia: What’s Next?
The DARAD Trial

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ABSTRACT

The main mechanism for the underlying pathogenesis of Alzheimer’s disease (AD) is the amyloid cascade hypothesis. A therapeutic agent that reduced amyloid deposition would be a disease modifying agent. Chlamydia pneumoniae has been suggested to have an etiologic role in AD. This finding raised the possibility of using long term antibiotics to prevent or treat AD. Doxycycline and rifampicin have been identified as potential disease-modifying agents. They are safe, inexpensive, and widely available agents. Besides their antimicrobial effects, they have non-antimicrobial properties which may account for their effects in the treatment of AD. These non-antimicrobial properties are anti-amyloidogenic, anti-tau, anti-inflammatory, anti-proteolytic, anti-oxidant, and metal ion chelator effects. To test the hypothesis that doxycycline and rifampicin reduce cognitive decline in AD patients, a pilot randomized controlled trial was conducted and the results showed significantly less decline in cognitive function. To test the efficacy of doxycycline and rifampicin in the treatment of AD, a multi-centre, randomized, controlled trial (DARAD Trial:
INTRODUCTION

Pathogenesis of Alzheimer’s Disease and Treatment Strategies

Alzheimer’s disease (AD) is a neurodegenerative disease that causes progressive cognitive deterioration, decline in activities of daily living, behavioral changes and neuropsychiatric symptoms. The pathological hallmarks of AD are amyloid beta deposition and tau protein abnormalities. The main mechanism for the underlying pathogenesis of the disease continues to be the amyloid cascade hypothesis. Clinical studies are ongoing to find therapies that target amyloid in AD.

Some possible therapeutic agents that would play a role in reducing amyloid beta deposition are inhibiting beta or gamma secretase inhibit amyloid beta aggregation and/or increase clearance of amyloid beta fibrils are being investigated. Possible therapeutic agents are immunotherapy, beta and gamma secretase inhibitors, alpha secretase stimulators, glycosaminoglycan mimetics, and tau kinase inhibitors. A therapeutic agent that reduced amyloid deposition would be a disease modifying agent.

Doxycycline and rifampicin have been identified as potential disease-modifying agents. To test their effects...
in the treatment of AD, a multi-centre, randomized, controlled trial examining the efficacy of the antibiotics doxycycline and rifampicin is being conducted in 14 centers in Canada. This trial, the DARAD Trial (Doxycycline and Rifampicin for Alzheimer’s Disease) examines the effect of these antibiotics on outcomes of AD patients. They are safe, inexpensive, and widely available agents.

**Chlamydia pneumoniae and Alzheimer’s Disease**

*C. pneumoniae*, a common respiratory pathogen has been suggested to have an etiologic role in AD (4). It has been isolated from AD brains and has been shown to induce Alzheimer-like plaque formation in the brains of BALB/c mice (4,6,8). Other studies conducted to detect Chlamydia pneumonia in brains of AD patients had conflicting results (4,9,10). These findings raised the possibility of using long term antibiotics to prevent or treat AD.

**Mechanisms of Doxycycline and Rifampicin That May Affect AD**

Doxycycline and rifampicin are used against *C. pneumoniae* and mycobacterial infections, respectively. Besides their antimicrobial effects, these antibiotics have nonantimicrobial properties which may account for their effects in the treatment of AD. The nonantimicrobial properties of doxycycline and rifampicin that may affect AD pathogenesis are their anti-amyloidogenic, anti-tau, anti-inflammatory (IL-1β, TNF-α, IL-4, IL-10), anti-proteolytic (MMP-2 and MMP-9), anti-oxidant, and metal ion chelator effects. Furthermore, they both penetrate the blood brain barrier. Both rifampicin and doxycycline interfere with the accumulation of amyloid beta peptide and formation of amyloid beta fibrils. Rifampicin inhibits aggregation of amyloid beta fibrils and neurotoxicity in vitro (11,12). This activity was correlated with rifampicin’s radical-scavenging ability of hydroxyl free radicals (11). Tetracyclines disassemble amyloid beta fibrils (13). Doxycycline also suppresses mutant tau production in transgenic mice (14). Based on this evidence showing inhibition of the formation of these toxic amyloid beta polymers, it was hypothesized that doxycycline and rifampicin could play a role in the treatment of AD as disease modifying agents.

**Clinical Trials of Doxycycline and Rifampicin**

**Pilot Randomized Controlled Trial**

To test the hypothesis that doxycycline and rifampicin reduce cognitive decline in AD patients; a randomized controlled trial was conducted between 1999 and 2001 (15). This study examined the effects of doxycycline and rifampicin in AD patients who were given three months of treatment. They were followed for one year, the last nine months off treatment. Assessments of cognition, activities of daily living (ADL), behavior and mood were performed at 3, 6, and 12 months. Cognitive function was the primary outcome. The results showed significantly less decline in Standardized Alzheimer’s Disease Assessment Scale –cognitive subscale (SADAS-cog) scores in the active treatment group compared to placebo at 6 months, and a statistically significant improvement in Standardized Mini-Mental State Examination (SMMSE) scores in the treatment group at 12 months. The change in SADAS-cog scores from baseline between rifampicin + doxycycline and placebo groups is shown in Figure 1. A statistically significant change in depression, behavior, caregiver burden, and ADL occurred at 3 months. These differences were not significant at six or 12 months. *C. pneumoniae* serology was not significantly different between groups and did not show any reduction after antibiotic therapy.

The results of this study suggest that these drugs slow the progression of clinical decline and have disease modifying effects. At present, it is unclear if this effect is due to doxycycline, rifampicin, or both. The possible mechanisms underlying this result may reduce neurofibrillary tangle formation and/or increase anti-inflammatory effects.

This study was underpowered and the effects on behavior and mood were transient. A further study with larger numbers, with a longer period of treatment, was undertaken. Blood and cerebrospinal fluid (CSF) is being taken to measure biomarkers to explore different effects and mechanisms of the treatment in this study.

**DARAD Trial**

This larger randomized controlled trial, funded by Canadian Institutes of Health Research (CIHR), is named the DARAD Trial (Doxycycline and Rifampicin for Alzheimer’s disease). Physicians’ Services Incorporated Foundation (PSI) provided additional funding to examine biomarkers in the CSF and serum, to explore the effects of doxycycline and rifampicin on these biomarkers.

The primary objective of this study is to determine the impact of rifampicin and doxycycline, over a one ye-
ar period, on cognition, function, mood, and behaviour. The central hypothesis of the DARAD trial is that doxycycline and/or rifampin have beneficial effects in patients with AD by acting as antiinflammatory agents by modulating inflammatory biomarkers in the blood and CSF and/or by modifying tau formation and/or changing the amyloid cascade. These effects will be tested by comparing the levels of inflammatory cytokines in serum and CSF between patients with AD and normal age-matched controls. The effects of these antibiotics on the biomarkers including amyloid beta 40 and 42 in the serum and CSF and total-tau (T-tau) and phosphorylated-tau (P-tau) in the CSF will also be tested to examine the mechanism of action of these drugs in AD.

The secondary objective is to determine if treatment with either doxycycline or rifampin alone is as efficacious as the combined treatment. A comparison of combined and single therapies versus placebo will determine if the effects are caused by one drug or the combination.

METHODS of DARAD TRIAL

Study Patients

The DARAD is a multi-centre, blinded, randomized, controlled trial comparing different regimens of the antibiotics doxycycline and rifampin in the treatment of AD. This study is ongoing with 14 centers in Canada participating. The target sample size is 500 patients with probable AD.

Inclusion criteria are: Age ≥ 50, probable AD, SMMSE score 14-26 inclusive, consenting patient, consenting caregiver, sufficient English to complete standardized testing in English, with stable health where the patient is reasonably expected to complete a 1 year trial.

Exclusion criteria are: Other neuro-degenerative diseases such as Lewy body, Parkinson’s, fronto-temporal, Huntington’s Chorea, Down’s Syndrome or Creutzfeld Jacob Disease; cognitive impairment due to acute cerebral trauma, subdural hematoma, injuries from chronic trauma, hypoxic cerebral damage; B12 deficiency, cancer or infections; endocrine deficiencies; hypercalcemia, hypothyroidism, hyperparathyroidism, Cushing’s syndrome, severe renal failure, poorly controlled diabetes mellitus, pituitary disease; mental retardation; significant cerebrovascular disease or multi-infarct dementia; intra-cranial pathology, tumour or hydrocephalus; history of epilepsy or convulsions; clinically significant psychiatric conditions or moderate to severe behavioural disturbances; clinically significant cardiac, hepatic, renal, pulmonary, metabolic or endocrine diseases; history of drug or alcohol abuse; history of myasthenia gravis; clinically significant cardiac disease such as cardiac surgery in the past six months, unstable angina or poorly controlled congestive heart failure, uncontrolled hypertension with systolic pressure greater that 180 mmHg or diastolic pressure greater...
that 110 mmHg anti-dementia treatments except donepezil, galantamine, rivastigmine, memantine, ASA up to 650 mg, vitamin E up to 400 IU, multi B vitamins, gingko biloba, Cox II inhibitors or statins (if doses are stable for at least three months and will be stable during the trial); other investigational drugs; long-term antibiotics; allergy to doxycycline or rifampicin.

**Clinical Outcomes**

Patients are treated for 12 months in four treatment arms:

1. Doxycycline + rifampicin,
2. Doxycycline + placebo-rifampicin,
3. Rifampicin + placebo-doxycycline,

Doxycycline is given in 100 mg twice a day and rifampicin 300 mg is taken once a day. Placebos matched to both doxycycline and rifampicin are used to maintain the blinding. The co-primary outcomes of the study are the Standardized Alzheimer’s Disease Assessment Scale-Cognitive Subscale (SADAS-cog), and the Clinical Dementia Rating scale (CDR). The SADAS-cog is an objective measure of cognition and the CDR is a measure of global function. The secondary outcomes are Standardized Mini-Mental State Examination (SMMSE), AB Cognitive Screen 100 (ABCS 100), Geriatric Depression Scale (GDS), activities of daily living (Lawton Scale), and Dysfunctional Behaviour Rating Instrument (DBRI). Clinical outcomes are assessed at 0, 3, 6, 9 and 12 months.

**CSF and Blood Biomarkers**

Blood analyses and CSF biomarkers will be measured before and after one year of treatment as a sub study of the DARAD trial to find out which markers doxycycline and rifampicin affect. CSF biomarkers will be used to follow the disease progress and to identify the mechanisms of these drugs’ effects in AD. Blood and 10 mL of CSF samples are obtained from 100 patients and analyzed for changes in biomarkers of AD before and after treatment. These biomarkers are amyloid beta (1-40), amyloid beta (1-42), P-tau, T-tau, APP isoforms (β-sAPP and α-sAPP), matrix metalloproteinases (MMP-2, MMP-9), metal ions (copper, iron, zinc, manganese), pro-inflammatory cytokines (IL-1β, TNF-α), and...
antinflammatory cytokines (IL-4 and IL-10). The correlation between the changes in CSF biomarkers and the clinical outcomes will be assessed. These observations will provide insight into the effects of doxycycline and rifampicin on AD pathology and clinical outcomes.

The CSF and serum biomarkers will also be compared with an age-matched control group, without AD. This correlation of biomarkers between non-AD and AD patients may help to identify a biomarker that could be used in the diagnosis and/or to monitor activity/progression/change of AD.

**Neuroimaging**

MRI using Susceptibility Weighted Imaging (SWI) will also be performed at baseline and after the one year of treatment in 100 patients. The changes in the neuroimaging findings will be correlated with the clinical and biological markers in blood and CSF.

**PROGRESS of DARAD TRIAL to DATE**

Fourteen centers in Canada are recruiting patients to DARAD trial. On February 3, 2009, 302 patients were enrolled in the study (Figure 2). The study drugs are well tolerated and currently about 90% of the patients complete 12 months of study treatment.

Within the CSF/blood sub study of the DARAD, 79 baseline and 38-12 month samples have been collected to date.

**CONCLUSION**

The DARAD trial will culminate 13 years of study into the effects of these antibiotics, as potential disease modifying agents, in AD. The drugs are well known, easily available and well tolerated. The trial will recruit another 150 patients in 2009 and 2010. The last patient will be through sometime in 2011. CSF analyses may be available sooner because these samples are blinded again with only treatments known. In this way they can be analysed even before the main clinical trials ends. We eagerly await these results to guide further inquiry and exploration.

**REFERENCES**


