Metal-Protein Attenuation With Iodochlorhydroxyquin (Clioquinol) Targeting Aβ Amyloid Deposition and Toxicity in Alzheimer Disease

A Pilot Phase 2 Clinical Trial

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Background: Alzheimer disease (AD) may be caused by the toxic accumulation of β-amyloid (Aβ).

Objective: To test this theory, we developed a clinical intervention using clioquinol, a metal-protein–attenuating compound (MPAC) that inhibits zinc and copper ions from binding to Aβ, thereby promoting Aβ dissolution and diminishing its toxic properties.


Results: Thirty-six subjects were randomized. The effect of treatment was significant in the more severely affected group (baseline cognitive subscale score of the Alzheimer’s Disease Assessment Scale, ≥25), due to a substantial worsening of scores in those taking placebo compared with minimal deterioration for the clioquinol group. Plasma Aβ42 levels declined in the clioquinol group and increased in the placebo group. Plasma zinc levels rose in the clioquinol-treated group. The drug was well tolerated.

Conclusion: Subject to the usual caveats inherent in studies with small sample size, this pilot phase 2 study supports further investigation of this novel treatment strategy using a metal-protein–attenuating compound.

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Clioquinol was withdrawn for oral use in 1970 because of its association with subacute myelo-optic neuropathy, possibly caused by vitamin B12 deficiency.3,8 We believed the drug should be reevaluated,9 and therefore prepared a double-blind phase 2 clinical trial. We chose a dose escalation schedule to maximize the chance of detecting a cognitive or biochemical effect while minimizing the risk for adverse effects. The study was powered to detect only conspicuous effects on cognition. The starting dosage of 3.3 mg/kg per day was within the same order of magnitude of the effective dosage in the mouse model.
Figure 1. Outline of the flowchart of subjects studied. BID indicates twice daily.

Herein we report the results demonstrating the efficacy of clioquinol treatment in producing effects on plasma Aβ and zinc ion (Zn²⁺) levels. In addition, the drug was well tolerated and inhibited cognitive decline in patients who, untreated, otherwise experienced deterioration.

METHODS

STUDY POPULATION

Criteria for participation in the study included informed consent (Consent to Special Procedures administered by the Victorian Civil and Administrative Tribunal, Melbourne, Victoria, and third-party consent); a diagnosis of probable AD by means of criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association; a cognitive subscale score on the Alzheimer's Disease Assessment Scale (ADAS-cog score) of 20 to 45; a Mini-Mental State Examination score of 10 to 24; and receipt of a 5- to 10-mg dose of donepezil for at least 6 months.

STUDY DESIGN

The study was a double-blind, placebo-controlled, parallel-group randomized design. Thirty-six patients and their caregivers were recruited. The duration of the study was 36 weeks. The oral dosage of clioquinol was 125 mg twice daily from weeks 0 to 12, 250 mg twice daily from weeks 13 to 24, and 375 mg twice daily from weeks 25 to 36.

STUDY PROCEDURES

Screening procedures consisted of a medical history, physical examination, psychometric tests, nerve conduction tests, and visual evoked responses. Blood was collected for apolipoprotein E typing and assays of metals and Aβ. All patients continued to receive donepezil and received intramuscular cyanocobalamin (vitamin B₁₂), 100 µg, every 4 weeks.

OUTCOME MEASURES

The primary efficacy variable was change from the baseline score on the ADAS-cog at weeks 4, 12, 24, and 36. Plasma Aβ, zinc, and copper levels were all measured every 4 weeks.

DOUBLE-ANTIBODY CAPTURE ENZYME-LINKED IMMUNOSORBENT ASSAY FOR Aβ DETECTION

Plates were coated with monoclonal antibody (mAb) G210 (for Aβ₁₋₄₀) or mAb G211 (for Aβ₁₋₄₂) and washed, and biotinylated mAb WO2 was added. Bound antibody was detected with streptavidin-labeled Europium (PerkinElmer, Inc, Melbourne, Victoria).

THERAPEUTIC DRUG MONITORING

At weeks 12, 24, and 36, clioquinol blood levels were assayed by means of high-performance liquid chromatography at the Centre for Pharmaceutical Research, University of South Australia, Adelaide.

SAFETY MEASURES

Standard adverse event reporting was conducted. Visual evoked responses and results of nerve conduction studies and a full ophthalmic examination were obtained at screening, at week 16, and before the final trial visit.

DATA ANALYSIS

Data management was undertaken by independent contractors (Kendle International Inc, Oakleigh, Melbourne, Victoria). Evidence of efficacy was indicated by a significant difference in change from baseline between treatment arms. Analysis of variance was the principal method of evaluating statistical significance. Differences between groups on categorical measures were analysed using exact statistical methods. The baseline illness severity factor was created, as planned, by division of the sample into 2 groups at the median ADAS-cog score at baseline, yielding less severely and more severely affected groups (n=8 and n=8, respectively, in the clioquinol treatment arm; and n=7 and n=9, respectively, in the placebo arm).

RESULTS

SUBJECT RECRUITMENT AND DEMOGRAPHICS

Thirty-six subjects were recruited, and 32 had sufficient data for per protocol analysis (Figure 1). The groups did not differ across relevant demographic, biological, and clinical variables at baseline (Table 1), other than the treatment arm having a higher mean premorbid IQ than the placebo group as estimated using the National Adult Reading Test (111.4 vs 104.9; t₁₆=4.40; P<.001). The National Adult Reading Test IQ and thyrotropin level were subsequently provisionally entered into analyses as covariates, but were found to be not significant in any analysis.
PROOF OF CONCEPT

Ideally, any drug targeting the Aβ pathway should have the following 2 principal effects: a disease-modifying effect as assessed by cognitive variables and a biological response assessed by measurement of Aβ levels in the blood, the cerebrospinal fluid, or the brain.

Changes in the ADAS-cog score at weeks 4, 12, 24, and 36 from baseline were subject to 2-way analysis of variance with factors of treatment arm and baseline illness severity. As planned in the protocol, the effect of severity of illness was examined by stratification of the sample into subjects less or more severely affected than the median score of the baseline ADAS-cog (<25 and ≥25). At baseline, there were no significant or near-significant differences between the main effect of the treatment arm (F1,28=0.21; P = .65). Similarly, there were no significant differences between treatment arms at either level of severity. The main effect of the treatment arm was not significant at any week, although trends toward significance were noted at weeks 4 (F1,28=3.55; P = .07) and 24 (F1,28=6.63; P = .02) (Figure 2A). Simple effects tests within the level of severity showed the main effect to be separable into nonsignificant results for the less-severe stratum on all weeks and significant differences in the more-severe stratum at weeks 4 (F1,28=7.73; P = .01) and 24 (F1,28=6.63; P = .02) (Figure 2B). This trend was maintained at week 36 but narrowly escaped statistical significance (F1,28=3.62; P = .07). The difference in mean change from baseline ADAS-cog score in the clioquinol arm compared with the placebo arm at weeks 24 and 36 was a difference of 7.37 (95% confidence interval, 1.51-13.24) and 6.36 (95% confidence interval, −0.50 to 13.23), respectively.

The Mini-Mental State Examination, a less sensitive measure of cognitive impairment, showed a similar pattern without reaching significance. By contrast, the noncognitive score on the ADAS and the Clinician Interview-Based Impression of Change did not show any clear differences or trends. Results of apolipoprotein E genotyping did not disclose any effect other than an over-representation of the ε4 allotype in both groups.
There were no significant differences in baseline plasma Aβ42/H9252 levels between treatment arms or severity strata. The plasma Aβ42/H9252 level declined significantly from baseline in the clioquinol-treated group from week 20 onward; during the same time, the plasma Aβ42/H9252 level in the placebo group increased (Figure 3A). Stratification by illness severity demonstrated that changes were evident only in the less severely affected group (Figure 3B). The wide variance in individual levels at baseline in plasma Aβ42/H9252 led to reduced power of the study to detect any significant differences in mean changes between groups.

Analysis of plasma Aβ42 levels showed overall similar trends, with significant differences between placebo and clioquinol groups observed at weeks 8, 32, and 36 in the less severely affected groups. For individuals, there was a highly significant (*P* < .001) correlation between Aβ42 and Aβ40 levels.

**EFFECT ON PLASMA ZINC AND COPPER LEVELS**

Administration of clioquinol was associated with a significant elevation of total plasma zinc level (Figure 4A) but with no effect on plasma copper level (Figure 4B). Samples collected with an indwelling catheter at weeks 12, 24, and 36 were found to be unreliable for technical reasons and were therefore omitted from this analysis. Mean absolute levels of zinc (61 µg/dL [9.4 µmol/L]) in all groups at baseline were below age-related normative values. Mean absolute levels of copper (83 µg/dL [13.1 µmol/L]) were within the age-related normative range. Correlation of plasma Aβ42/40 levels with zinc and copper levels assayed on the same or on subsequent occasions showed no significant associations.

**BLOOD LEVELS OF CLIOQUINOL**

Mean ± SD steady-state predose levels of clioquinol at total daily dosages of 250, 500, and 750 mg were 4.03 ± 2.10, 6.74 ± 3.70, and 7.60 ± 2.15 µg/mL, respectively.
SAFETY RESULTS AND ANALYSIS

Safety analysis was conducted on all data irrespective of the stage reached in the trial. There were a total of 123 attributable adverse events reported, 64 in the treatment group and 59 in the placebo group. Of the attributable adverse events with a risk of greater than 10% or where the point estimate risk ratio was greater than 2.0 or less than 0.5, the mean number of discrete events per subject was not significantly different between arms (Table 2). Serious adverse events developed in 5 subjects. Impaired visual acuity and color vision (in the absence of other neurological signs or symptoms) developed in a 66-year-old woman with hypertension, hyperlipidemia, and a history of glaucoma and visual migraine during weeks 31 to 36 of the trial, while she was receiving clioquinol, 375 mg twice daily. This event was considered to be possibly attributable to clioquinol, and her symptoms resolved on treatment cessation. The following 4 nonattributable serious adverse events were recorded: 1 death due to intracranial hemorrhage (placebo group) and 3 hospitalizations for hip pain (placebo group), syncope due to impaired cardiac function (clioquinol group), and confusion (placebo group).

The findings support a proof of concept in humans that a drug targeting metal-\(\alpha\)/H\(_{\beta2}\) interactions can have a significant effect on \(\alpha\)/H\(_{\beta2}\) metabolism and, through this, a beneficial modification on the progression of AD. The clinical benefit of clioquinol in this study population was only seen in the more severely affected subjects, probably due to the low power of the study and the nonlinear sensitivity of the ADAS-cog instrument to detect relatively slight cognitive differences in the less severely affected groups (Figure 5). The separation of 3 ADAS-cog points achieved after 24 weeks of treatment with the acetylcholinesterase inhibitor, donepezil, required a study population of more than 300 subjects.10 The significant benefit seen in the more severely affected treatment group at 4 weeks is also of interest, as this may represent the short-term effect of clioquinol neutralizing the neurotoxicity of the soluble pool of \(\alpha\)/H\(_{\beta2}\).11

The data showing a significant lowering of plasma \(\alpha\)/H\(_{42}\) levels are more compelling (Figure 3). The relationship between plasma \(\alpha\)B level and ADAS-cog scores has not yet been determined, but is probably nonlinear.

### Table 2. Attributable Adverse Events With a Risk of Greater Than 10% in Either Arm or Where Point Estimate Risk Ratio Is Greater Than 2.0 or Less Than 0.5

<table>
<thead>
<tr>
<th>Category</th>
<th>Clioquinol Treatment Group (n = 16)</th>
<th>Placebo Group (n = 16)</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postural hypotension</td>
<td>12</td>
<td>11</td>
<td>1.09 (0.67-1.79)</td>
</tr>
<tr>
<td>Postural tachycardia</td>
<td>12</td>
<td>8</td>
<td>1.33 (0.74-2.40)</td>
</tr>
<tr>
<td>Postural dizziness</td>
<td>7</td>
<td>3</td>
<td>2.33 (0.71-7.63)</td>
</tr>
<tr>
<td>Subjects with (\geq 1) postural symptom</td>
<td>13</td>
<td>14</td>
<td>0.93 (0.64-1.36)</td>
</tr>
<tr>
<td>Neurological</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impaired nerve conduction</td>
<td>3</td>
<td>1</td>
<td>3.0 (0.34-26.2)</td>
</tr>
<tr>
<td>Impaired reflexes</td>
<td>1</td>
<td>2</td>
<td>0.5 (0.05-5.04)</td>
</tr>
<tr>
<td>Numb legs</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Subjects with (\geq 1) symptom</td>
<td>6</td>
<td>4</td>
<td>1.5 (0.51-4.43)</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1</td>
<td>4</td>
<td>0.25 (0.03-2.02)</td>
</tr>
<tr>
<td>Constipation</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>2</td>
<td>1</td>
<td>2.0 (0.2-20.1)</td>
</tr>
<tr>
<td>Subjects with (\geq 1) symptom</td>
<td>5</td>
<td>4</td>
<td>1.25 (0.4-3.91)</td>
</tr>
<tr>
<td>Renal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>5</td>
<td>5</td>
<td>1.00 (0.35-2.87)</td>
</tr>
<tr>
<td>Hematological</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Liver function tests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raised (\gamma)GT level</td>
<td>2</td>
<td>1</td>
<td>2.0 (0.2-20.1)</td>
</tr>
<tr>
<td>Raised bilirubin level</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Subjects with (\geq 1) abnormal result</td>
<td>4</td>
<td>1</td>
<td>4.0 (0.49-32.4)</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased vitamin B(_{12}) level</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Mean (SD) No. of discrete adverse events per subject</td>
<td>3.38 (2.14)</td>
<td>2.78 (1.48)</td>
<td>0.611 (−0.64 to 1.89)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; \(\gamma\)GT, \(\gamma\)-glutamyltransferase; RR, relative risk.

*Unless otherwise indicated, data are expressed as number of subjects.
†Expressed as mean difference (95% CI) (\(P = .33\)).
An important result of clioquinol treatment of subjects with AD is the elevation in plasma zinc levels (Figure 4A). Experimental studies of clioquinol on the mouse brain showed similar increases of zinc level of 13% and copper level of 19%. This finding also indicates that, in contrast to a typical metal chelator such as desferrioxamine, the MPAC drugs (of which clioquinol is but one example) are not gross tissue chelators. The affinity of clioquinol for copper and zinc ions is relatively modest (approximately nanomolar) and is likely to be facilitating their dissociation from the lowest-affinity metal binding sites on Aβ.

The measured basal levels of plasma clioquinol in the current study ranged from 13 to 25 µmol/L (+-8 µg/mL). After allowing for a large proportion of clioquinol being bound to protein, the available active compound in the brain should be 100 to 200 nmol/L. The concentration of total Aβ in the brain with AD varies considerably, but is estimated to range from the high nanomolar to the low micromolar range, of which less than 1% is available as a toxic soluble species. Actual measurements of extracellular brain clioquinol levels will be required, together with plasma pharmacokinetics, before a more rational approach to dosing can be applied.

Safety issues are of concern in a study involving the chronic administration of a drug with a history of adverse events. We balanced the risks of treating a malignant disease such as AD against the relatively low risk for development of subacute myelo-optic neuropathy by careful monitoring and by ensuring complete normality of vitamin B₁₂ and folate metabolism. Clioquinol-associated optic neuropathy was suspected in 1 subject with a prominent history of eye disease, but a direct causal link to clioquinol remains uncertain, given that disturbances of color vision and other ophthalmologic changes occur during the natural history of AD. Twenty-seven subjects have now been receiving this drug at a dosage of 500 to 750 mg/d for more than 18 months. No clioquinol-attributable adverse events have developed in any of these subjects.

### CONCLUSIONS

The safety profile and biochemical efficacy of clioquinol in this population are sufficiently encouraging to allow for future trials to take this investigation of a novel therapeutic intervention (clioquinol itself or a pharmacologically improved backup) targeting Aβ amyloid to the next phase. This class of MPAC may also be considered for related conditions such as Parkinson disease, in which α-synuclein and iron could interact in a manner analogous to Aβ and zinc and copper ions.

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From the Departments of Pathology (Drs Ritchie, Bush, Macfarlane, Cherry, Li, Tammer, and Masters; Mss Mastwyk, Mavros, and Volitakis; and Mr Carrington), Medicine (Drs MacGregor, Kiers, and Davis), and Psychiatry (Dr Ames), The University of Melbourne, The Mental Health Research Institute of Victoria, Parkville, Victoria (Drs Ritchie, Bush, Mackinnon, Macfarlane, Cherry, Li, Tammer, and Masters; Mss Mastwyk, Mavros, and Volitakis; and Mr Carrington);
the Department of Psychiatry and Behavioural Science, Royal Free Campus, University College London, London, England (Dr Ritchie); the Department of Psychological Medicine, Monash University, Clayton, Victoria (Dr Mackinnon); the Department of Neurology, Royal Melbourne Hospital, Parkville, Victoria (Drs MacGregor, Kiers, and Davis); the Institute of Clinical Neuroscience, Göteborg University, Göteborg, Sweden (Dr Xilinas); the Center for Molecular Biology, University of Heidelberg, Heidelberg, Germany (Dr Beyreuther); and the Genetics and Aging Research Unit, Massachusetts General Hospital, Boston (Drs Bush and Tanzi). Dr Ritchie has been paid for his time and effort in analyzing the results from the trial sponsor, Prana Biotechnology. Drs Bush and Tanzi are paid consultants, are on the Scientific Advisory Committee, and are stockholders of Prana Biotechnology and had no input into the drafting or review of any portion of the manuscript other than those portions pertaining to study concept and design and consultation on biochemistry and no input into the analysis of the data from the other portions. Drs Macfarlane and MacGregor and Ms Mastwyk were employed by the Mental Health Research Institute through funds provided by Prana Biotechnology. Dr Cherny is a stockholder in Prana Biotechnology. Dr Xilinas has financial and intellectual interests in the development of clioquinol. Dr Beyreuther is on the Scientific Advisory Committee of Prana Biotechnology. Dr Masters is a director of Prana Biotechnology, chairperson of its Scientific Advisory Committee, and a stockholder.

Author contributions: Study concept and design (Drs Ritchie, Bush, Mackinnon, Xilinas, Ames, Davis, Tanzi, and Masters and Ms Mastwyk); acquisition of data (Drs Macfarlane, MacGregor, Kiers, Li, Tammer, Ames, and Masters; Mss Mastwyk, Mavros, and Voltiakis; and Mr Carrington); analysis and interpretation of data (Drs Ritchie, Mackinnon, Macfarlane, MacGregor, Kiers, Cherny, Xilinas, Ames, Beyreuther, and Masters); drafting of the manuscript (Drs Ritchie, Mackinnon, Tammer, and Masters and Ms Mastwyk); critical revision of the manuscript for important intellectual content (Drs Ritchie, Bush, Mackinnon, Macfarlane, MacGregor, Kiers, Cherny, Li, Xilinas, Ames, Davis, Beyreuther, Tanzi, and Masters; Mss Mastwyk, Mavros, and Voltiakis; and Mr Carrington); statistical expertise (Drs Ritchie, Mackinnon, and Davis); obtained funding (Drs Bush and Masters); administrative, technical, and material support (Drs Ritchie, Bush, Macfarlane, MacGregor, Cherny, Li, Tammer, Ames, Beyreuther, and Masters; Mss Mastwyk, Mavros, and Voltiakis; and Mr Carrington); study supervision (Drs Ritchie, Bush, Macfarlane, Kiers, Cherny, Li, Ames, and Masters); consultation on biochemistry (Drs Bush, Beyreuther, and Tanzi).

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REFERENCES