Olive polyphenols and bioavailable glutathione: Promising results in patients diagnosed with mild Alzheimer’s disease

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Abstract

Introduction: Recent studies highlighted the role of olive polyphenols in disrupting the ordered structure of highly cytotoxic amyloid beta protofibrils and the efficacy of a derivatized form of glutathione to counteract neuronal oxidative stress affecting specific brain regions at early stages of Alzheimer’s disease (AD) pathogenesis. We performed a randomized cross-over clinical trial to evaluate their potential benefits in mild AD.

Methods: Oleuropein and S-acetyl glutathione were administered as dietary supplement for 6 months to 18 patients diagnosed for probable mild AD according to International Working Group 2 criteria. Patients underwent an extensive cognitive and behavioral neuropsychological test battery at the beginning and end of the study to evaluate cognitive deterioration, memory, visuospatial abilities, attention, language, executive functions, and behavioral disorders. We compared patients receiving treatment to patients receiving no treatment.

Results: All the measured neurocognitive parameters stabilized or improved after the treatment in all patients.

Discussion: Dietary supplement with olive polyphenols and bioavailable glutathione could be useful for patients diagnosed with mild AD.

Keywords
Alzheimer disease, glutathione, olive polyphenols

1 | INTRODUCTION

According to Alzheimer’s Disease International, Alzheimer’s disease (AD) is characterized by the development of amyloid beta (Aβ) plaques and tau neurofibrillary tangles in the brain.1 The great majority of clinical trials of potential disease-modifying therapies for AD (including statins, non-steroidal anti-inflammatory drugs, monoclonal antibodies, estrogens, and nerve growth factor) have yielded substantially negative results over the past 20 years. Furthermore, in spite of genetic and molecular evidence pointing to Aβ as a key player in AD pathogenesis,2 most trials with anti-amyloid therapies have failed, possibly due to over-simplistic pathogenic constructs unfit to cope with the complexity and variety of the pathological modifications favoring the expression of the clinical phenotype, including neuronal loss.3 Numerous longitudinal studies using several AD biomarkers indicated that the AD pathology develops decades before symptom appearance,4 suggesting the usefulness of preventive multi-target treatments aimed at hindering or delaying disease onset.

Recent data indicate that deglycated oleuropein (Ole), the most abundant polyphenol in virgin olive oil, interferes with amyloid precursor protein and tau protein processing, preventing toxic oligomer growth both in vitro, in a transgenic strain of Aβ-expressing...
C. elegans, and in CRND8 transgenic mice, a model of Aß plaque deposition. In particular, transgenic mice fed a normal diet supplemented with Ole showed a dose-dependent protection against cognitive deterioration compared to normally fed littermates. At the tissue level, these mice displayed a significant improvement of synaptic function, plaque load, neurogenesis, and neuroinflammation.

Glutathione, the main antioxidant defense in the body, is a metabolite with one of the highest concentrations in mammalian tissue cells. Its concentration (between 1 and 10 mM depending on the cell type) decreases with age and in specific central nervous system regions in AD patients.

When taken orally, glutathione as such is unable to cross the alimentary canal intact as it undergoes a hydrolytic cleavage catalyzed by digestive enzymes, in particular by pancreatic peptidase and the intestinal wall. In recent years, S-acetyl glutathione (SAG) and glutathione derivatives conjugated with long-chain fatty acid conjugates have been shown to be highly bioavailable from preclinical and clinical studies. These glutathione derivatives have proven to be capable of significantly increasing the intracellular concentration of the tripeptide even when supplied at concentration levels of orders of magnitude lower than those reached within the cells. This effect is believed to be the result of a trapping process by cells, which do not allow the release of glutathione penetrated as thioesters and subsequently hydrolyzed by intracellular thioesterases. In particular, glutathione derivatives with omega-3 fatty acids such as S-linolenoyl glutathione have proven particularly effective in counteracting oxidative stress in the neurons of patients with AD and in preventing cell death in cultures of human neurons exposed to oxidative stress induced by hydrogen peroxide and Aß42.

The association with a bioavailable form of glutathione is therefore expected to further ameliorate Ole neuroprotection by counteracting oxidative stress, a dishomeostasis not directly relieved by Ole in TgCRND8 mice. Even if the possible therapeutic role of many antioxidants in AD has been deeply investigated, no literature yet ascribes the potential benefits of these two molecules for patients who have already developed AD.

On the basis of the above-reported findings from preclinical studies, we performed a randomized cross-over clinical trial by enrolling patients with mild AD diagnosis to which a nutraceutical formulation comprising 50 mg/cps SAG and 80 mg/cps oleuropein was administered b.i.d. Exclusion criteria included previous obtainment of informed consent, in a 12-month trial and randomly assigned to two groups of equal sizes: Group 1, receiving treatment in the first 6 months and no treatment for the second 6 months, and Group 2, receiving no treatment for the first 6 months, and treatment for the second 6 months. Treatment consisted of a nutraceutical formulation comprising 50 mg/cps SAG and 80 mg/cps oleuropein associated with vitamin B6 1 mg/cps, vitamin B12 3mcg/cps, vitamin E 15 IU cps, vitamin D3 4 mcg/cps, piperine 3 mg/cps, bacopa dry extract 100 mg/cps administered b.i.d. Exclusion criteria included previous phenomena of intolerance/allergy to one of the components present in the formulation. All the patients should have undergone a complete set of neuropsychological tests to evaluate cognitive functions and behavioral disturbances at the moment of the enrollment (T0), after 6 months (T1), and after 12 months (T2); cognitive deterioration, memory, visuospatial abilities, attention, language, executive functions, and behavioral disorders were included in the study as outcome variables using 14 indicators (Table 1). Given the nature and size of the study, a difference-in-difference (DID) approach was used to evaluate outcome variables. DID is a quasi-experimental design that makes use of longitudinal data from treatment and control groups to obtain an appropriate counterfactual to estimate a causal effect.

The effect of the treatment (having taken the food supplement for 6 months) was calculated by comparing the average change in the outcome variables for the treatment group to the average change for the control group. We used a t test of the difference between T0 and T1 for the two groups (intergroup comparison). We did not use intragroup tests.

The study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans and was approved by the Italian Regional Ethics Committee. It provided for up to 40 patients diagnosed with mild AD according to the International Working Group-2 (IWG-2) criteria to be enrolled, previous obtainment of informed consent, in a 12-month trial.
TABLE 1  Groups’ demographic data and effect of treatment on outcomes variables

| Test                  | Group 1 (Treatment) | Group 2 (Control) | Difference | Difference | Diff-in-diff | P    | SE   |
|-----------------------|---------------------|-------------------|------------|------------|-------------|------|------|
| Cognitive deterioration | MMSE (0–30)         | 21.20 (1.03)      | 22.90 (1.20) | 1.70 (+8%)  | 22.13 (0.83) | 22.13 (0.83) | 0.00 (+0%) | 1.70 .0008 0.4125 |
|                       | CDT (0–9)           | 5.40 (1.84)       | 6.20 (1.69)  | 0.80 (+15%) | 4.75 (1.28)  | 4.25 (1.04)  | 0.50 (−11%) | 1.30 .0135 0.4684 |
| Memory                | RAVLT IR (0–75)     | 25.20 (5.32)      | 26.90 (4.68) | 1.70 (+7%)  | 24.75 (2.12) | 22.25 (2.25) | 2.50 (−10%) | 4.20 .0014 1.0875 |
|                       | RAVLT DR (0–15)     | 0.30 (0.67)       | 1.20 (1.23)  | 0.90 (+300%)| 0.63 (0.92)  | 0.13 (0.35)  | 0.50 (−80%) | 1.40 .0046 0.4259 |
|                       | RCF IR (0–36)       | 2.40 (2.80)       | 4.60 (3.66)  | 2.20 (+92%) | 1.50 (2.33)  | 0.75 (2.12)  | 0.75 (+50%) | 2.95 .0016 0.7785 |
| Visuospatial abilities | RCF C (0–36)        | 23.30 (6.45)      | 26.50 (5.15) | 3.20 (+14%) | 24.63 (4.50) | 24.50 (4.72) | 0.13 (−1%)  | 3.33 .0603 1.6452 |
| Attention             | MA (0–60)           | 32.00 (6.32)      | 32.90 (5.95) | 0.90 (+3%)  | 38.25 (7.69) | 35.00 (5.95) | 3.25 (−8%)  | 4.15 .0028 1.1763 |
| Language and speech   | AAT (0–120)         | 100.60 (5.46)     | 104.40 (3.92) | 3.80 (+4%)  | 103.38 (10.39) | 103.38 (10.39) | 0.00 (+0%) | 3.80 .0142 1.3809 |
| Executive functions   | FAB (0–18)          | 11.80 (1.93)      | 15.10 (0.99) | 3.30 (+28%) | 10.88 (2.23) | 9.25 (1.83)  | 1.63 (−15%) | 4.93 <.0000 0.5806 |
|                       | STEP (0–40)         | 21.50 (5.59)      | 24.90 (4.43) | 3.70 (+17%) | 24.00 (4.31) | 21.75 (4.83) | 2.25 (−9%)  | 5.95 <.0000 0.9605 |
|                       | SVF (0–∞)           | 32.50 (5.06)      | 35.80 (4.18) | 3.30 (+10%) | 31.38 (5.10) | 29.50 (3.93) | 1.88 (−6%)  | 5.18 <.0000 0.9107 |
|                       | PVF (0–∞)           | 24.90 (5.78)      | 30.50 (6.70) | 5.60 (+22%) | 27.75 (5.52) | 23.63 (4.90) | 4.13 (−15%) | 9.73 <.0000 1.4194 |
| Behavioral disorders  | NPI (0–144)         | 10.70 (5.58)      | 5.80 (3.94)  | 4.90 (−46%) | 12.00 (7.09) | 12.50 (6.65) | 0.50 (+4%)  | −5.40 <.0001 1.0797 |
|                       | AES (0–72)          | 40.70 (10.72)     | 33.60 (8.58) | 7.10 (−17%) | 33.63 (16.49) | 36.13 (15.68) | 2.50 (+7%)  | −9.60 <.0000 1.7221 |

**Abbreviations:** AAT, Aachener Aphasie Test; AChEI, acetylcholinesterase inhibitor; AES, Apathy Evaluation Scale; CDT, Clock Drawing Test; FAB, Frontal Assessment Battery; MA, Attentive Matrices; MMSE, Mini Mental State Examination; NPI, Neuropsychiatric Inventory; PVF, Phonological Verbal Fluency Test; RAVLT DR, Rey Auditory Verbal Learning Test–Delayed Recall; RAVLT IR, Rey Auditory Verbal Learning Test–Immediate Recall; RCF C, Rey Complex Figure–Copy; RCF IR, Rey Complex Figure–Immediate Recall.

summarized in Table 1. The effect of the treatment is statistically significant for all 14 outcome variables, and 11 of them are significant at the 1% level. These results appear surprisingly robust on a statistical basis, given the small sample size. In particular, the treatment seems to have a very strong and significant effect on:

1. Cognitive deterioration: Strong reduction of cognitive deterioration for treated group versus control, with Mini-Mental State Examination (MMSE) improvements of 8% for treatment group (vs. a 0% change for control group), significant at the 1% level and very near to the 2 points reported as the MMSE – minimal clinically important difference (MCID); and a Clock Drawing Test improvement of 15% for treatment group (vs. a 11% reduction for control group), with a P-value of .0135.

2. Improving memory: All three indicators are significant at 1% level; Rey Auditory Verbal Learning Test–Immediate Recall, Rey Auditory Verbal Learning Test–Delayed Recall, and Rey Complex Figure–Immediate Recall show an improvement of 7%, 300%, and 92%, respectively, for treatment group, compared to a deterioration of 10%, 80%, and 50% for control group (Figure 1).

3. Spatial and visual abilities: Improvement of 14% in Rey Complex Figure–Copy exam for treatment group (vs. a reduction of 1%, 80%, and 50% for control group).

4. Improving attention: Patients in the treatment group show an improvement of 3% in the Attentive Matrices, compared to a reduction of 8% in control group; this is significant at the 1% level.

5. Language and speech: An improvement of 4% in the Aachener Aphasie Test for treatment group (vs. a 0% change for the control group), with a P-value of .0603.

6. Improving executive functions: All indicators are significant at the 1% level. Improvements for treatment group are substantially and vary from a 10% improvement in Semantic Verbal Fluency Test (vs. 6% reduction in control group), to a 28%
improvement in the Frontal Assessment Battery for the treatment group (vs. 15% decrease in control group)

(7) Diminishing behavioral disorders: Strong reduction in treatment group compared to control group. Treatment group exhibits a reduction of 46% in the Neuropsychiatric Inventory (vs. an increase of 4% in control), and a reduction of 17% in the Apathy Evaluation Scale (vs. an increase of 7% in control). Both outcome variables are statistically significant at the 1% level.

4 | DISCUSSION

Overall, the treatment seems to successfully diminish the effect of neurodegeneration in all neuropsychological outcome variables observed. Even if the number of patients is very small and there is no placebo control, these relevant effects are unexpected and are tentatively ascribed by authors to a synergistic effect of the active compounds of the nutraceutical formulation toward neurotoxic activity of amyloid protofibrils and neuronal oxidative stress, two hallmarks in early stages of AD pathogenesis. Reviewing literature about this topic, we considered the SAG and Ole interaction as primarily responsible for the proposed metabolic synergy.20

Despite the “proof-of-concept” character of this study, the authors believe that the results are impactful considering the lack of therapeutic perspectives for AD. We are planning to perform a double-blind randomized controlled trial with a larger number of patients including neuropsychological measures and other validated AD biomarkers. At the moment, there is encouraging preliminary evidence that the described treatment could be profitably adopted for patients with mild cognitive impairment or mild AD.

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CONFLICTS OF INTEREST

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