Tolerability and pharmacokinetics of oxaloacetate 100 mg capsules in Alzheimer’s subjects

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1. Introduction

Alzheimer’s disease (AD) is clinically characterized by cognitive decline [1]. While hypotheses postulate its potential causes and propose various therapeutic targets, no clearly effective disease-modifying interventions are currently recognized.

The single greatest AD risk factor is advancing age. Brain bioenergetic function and mitochondrial integrity decline with advancing age and to a further extent when AD is present [2]. Energy metabolism-associated changes in AD include decreased glucose utilization, as indicated by fluoro-deoxyglucose positron emission tomography (FDG PET) studies that reliably reveal early and neuroanatomically predictable hypometabolic brain regions [3–5]. Activities of several mitochondria-localized enzymes, including enzymes of the Krebs cycle and the respiratory chain, are also reduced in AD subject brains and in some cases even peripheral tissues [2]. Some brain regions show an overall reduction in the number of normal-appearing mitochondria, apparently increased mitochondrial debris in autophagosomes, and low levels of the mitochondrial biogenesis-promoting peroxisome proliferator-activated receptor gamma coactivator (PGC1α) protein [6,7]. Some AD investigators believe energy metabolism, functional and structural changes may contribute to the progression of disease and perhaps even initiate it, and constitute reasonable therapeutic targets [8,9].

We previously reported changes in bioenergetic fluxes and infrastructure when cells or animals are exposed to various energy metabolism pathway intermediates. One immediate we evaluated is oxaloacetate (OAA), a dicarboxylic acid found in Krebs cycle and gluconeogenesis fluxes. Administering OAA to cultured neuronal SH-SY5Y cells enhances glycolysis and respiratory fluxes, increases PGC1α mRNA and protein, and increases mRNA and protein levels of a mitochondrial DNA (mtDNA)-encoded cytochrome oxidative (COX) subunit [10]. The brains of mice that received a two-week course of intraperitoneal (IP) OAA showed increased levels of PGC1α mRNA, an increase in the nuclear to cytosolic PGC1α protein ratio, and higher amounts of the COX subunit 4 protein [11]. Compared to saline injected
mice, the brains of the OAA-treated mice also showed higher hippocampal neurogenesis activity and changes suggesting enhanced brain insulin signaling and reduced neuroinflammation [11]. For these reasons we want to determine the effects of OAA on persons with AD.

2. Methods

This study was approved by the Kansas University Medical Center Human Subjects Committee and informed consent was obtained for all subjects. We recruited six AD subjects from the University of Kansas Alzheimer’s Disease Center (KU ADC) Clinical Core cohort; APOE genotype status and clinical dementia rating scale (CDR) scores are independently acquired for KU ADC cohort members. Subjects met the McKhann et al. AD criteria [12], had CDR scores of 0.5 or 1, and had dependently acquired for KU ADC cohort members. Subjects met the genotype status and clinical dementia rating scale (CDR) scores are in-

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For each visit subjects presented at 8 AM to the Clinical Trials Unit of

the homeostatic model assessment of insulin resistance (HOMA-IR) values for each subject using the following equation: (glucose in mg/dl x insulin in mcu/ml)/405. Initial and final visit HOMA-IR, weight, plasma amino acid, and cognitive score values were compared using a paired t-test approach. CBC, electrolyte, and LFT studies were analyzed for the appearance of clinical abnormalities.

3. Results

Subject characteristics are shown in Table 1. Mean age (with standard deviation) was 76.2 ± 8.2. Only one subject lacked an APOE4 allele. All of the subjects completed the study. During the course of the study no adverse events, treatment-induced symptoms, or clinically significant changes in safety labs were observed. Compliance estimates for the six subjects ranged from 76 to 100% (Table 2).

Weight, fasting glucose, fasting insulin, and HOMA-IR values for sub-
jects varied between visits, but as a group no consistent changes in weight or HOMA-IR were observed during the course of the study (Table 3). Although there was not a statistically significant reduction in post-treatment fasting glucose levels, post-treatment fasting glucose levels were slightly lower than pre-treatment levels in 5 of the 6 subjects. MMSE and ADASCog values also varied between visits, but as a group no consistent changes in the MMSE or ADASCog scores were observed during the course of the study (Table 3). The average visit 1 and visit 2 MMSE scores (with standard deviations) were, respectively, 21.2 ± 4.7 and 21.5 ± 5.1 and 19.5 ± 5.9. The average visit 1 and visit 2 ADASCog scores were 21.5 ± 5.1 and 23.7 ± 6.6 (Table 3).

Table 1
Subject characteristics.

| Subject | Age | Sex | APOE | CDR |
|---------|-----|-----|------|-----|
| 1       | 64  | M   | 4/4  | 0.5 |
| 2       | 68  | M   | 3/4  | 1   |
| 3       | 78  | M   | 3/3  | 1   |
| 4       | 83  | F   | 3/4  | 0.5 |
| 5       | 82  | F   | 3/4  | 1   |
| 6       | 82  | M   | 3/4  | 1   |
Quantitative plasma amino acid profiles were performed by Mayo Clinical Labs and included measurements of 42 amino acids and derivatives. Values from the visit 1 and 2 values were compared, and using a p value cut-off of 0.05 no statistically significant inter-visit differences were observed (Table 4). Values for two amino acids (glutamate and aspartate) most directly linked to OAA metabolism (as glutamate reacts with OAA to form aspartate and α-ketoglutarate) did not show statistically significant inter-visit changes. Levels of alanine, which is in equilibrium with pyruvate (a decarboxylation product of OAA), also did not change across the visits (Table 4).

For PK measurements performed using the enzymatic-based OAA quantification kit, the more sensitive fluorescence-based approach was confounded either by plasma auto-fluorescence at the critical excitation/emission wavelengths or else through a non-specific, activating interaction between plasma and kit components. No useful data were obtained using this approach. The kit’s absorbance-based approach was therefore utilized. The absorbance method has a ten-fold reduction in sensitivity as compared to the fluorescence assay, and the levels of OAA detected were very near the limit of detection for the method. Qualitative review of some of the data from this analysis indicates a possible Tmax peak occurs between 1 and 1.5 h after ingestion of a 100 mg capsule (data not shown). This pattern, though, was not consistently observed across subjects.

The attempts to measure OAA by the coupled enzyme assay consumed the acquired plasma samples from subject 2. Plasma samples for the remaining 5 subjects were analyzed by LC–MS/MS for both OAA and pyruvate. OAA concentrations for subject 1 were at or below our limit of quantitation (10 ng/ml), so the results from that patient were not included in the determination of PK parameters. All subjects showed quantifiable pyruvate concentrations.

Representative PK data are shown in Fig. 2. The plasma concentration versus time plots in the figure does not show typical PK behavior. The pre-dose OAA concentrations were about 60% of the observed Cmax for the first dose of OAA, and the pre-dose concentration and Cmax were virtually identical for the Day 28 dose. Neither day exhibited the expected absorption and clearance phases in the time course. It is important to note that the Tmax for OAA was centered on 1.2 h after ingestion of a 100 mg capsule (data not shown). This pattern, though, was not consistently observed across subjects.

The 100 mg dose did not yield consistent changes in either OAA or pyruvate levels. Our ability to detect consistent changes was likely hindered by high background levels of OAA and pyruvate, which may have obscured genuine treatment-induced changes. Regardless, our data suggest 100 mg of OAA does not significantly change an individual AD subject’s plasma level. Failure to detect any reliable treatment-induced amino acid changes is potentially consistent with but does not prove this possibility.

### Table 2
Compliance and safety.

| Subject | Compliance | New symptoms | Clinically significant abnormal safety labs |
|---------|------------|--------------|--------------------------------------------|
| 1       | 89%        | None reported| None                                        |
| 2       | 98%        | None reported| None                                        |
| 3       | 100%       | None reported| None                                        |
| 4       | 82%        | None reported| None                                        |
| 5       | 76%        | None reported| None                                        |
| 6       | 98%        | None reported| None                                        |

### Table 3
Weight, fasting glucose, fasting insulin, HOMA-IR, MMSE, and ADASCog data. V1 = visit 1; V2 = visit 2.

| Subject | Weight (kg) | Glucose | Insulin | HOMA-IR | MMSE | ADASCog |
|---------|-------------|---------|---------|---------|------|---------|
|         | V1 V2       | V1 V2   | V1 V2   | V1 V2   | V1 V2 | V1 V2   |
| 1       | 72.9 74.3   | 105 104 | 7.2 6.9 | 1.87 1.77 | 28 28 | 15 18   |
| 2       | 86.2 83.5   | 107 101 | 5.5 14.6 | 6.73 3.64 | 21 18 | 23 28   |
| 3       | 78.3 81.1   | 104 100 | 21.2 24.1 | 5.44 5.95 | 25 25 | 17 14   |
| 4       | 70.0 68.1   | 99 91   | 6.4 5.5 | 1.56 1.24 | 18 12 | 26 28   |
| 5       | 88.4 87.6   | 140 124 | 10.0 10.0 | 6.7 3.46 | 7.05 5.15 | 18 28 | 23 28   |
| 6       | 76.4 78.1   | 100 105 | 2.8 3.9 | 0.69 1.01 | 20 16 | 20 31   |

### Table 4
Plasma amino acid measurements. V1 = visit 1; V2 = visit 2.

| Subject | Glutamate | Aspartate | Alanine |
|---------|-----------|-----------|---------|
|         | V1 V2     | V1 V2     | V1 V2   |
| 1       | 29 45     | 1 3       | 451 343 |
| 2       | 74 51     | 4 3       | 562 617 |
| 3       | 50 49     | 2 3       | 326 479 |
| 4       | 20 16     | 2 2       | 261 285 |
| 5       | 27 33     | 2 2       | 474 374 |
| 6       | 36 43     | 2 2       | 338 407 |

**Fig. 2.** OAA plasma concentration versus time. Results are shown for the analysis of plasma samples from subject 3.
Table 5

| Compartment | Cmax, ng/ml | Tmax, h | Cmax/C0 |
|-------------|------------|---------|---------|
| OAA (n = 8) | 551 ± 241 (210–1122) | 1.2 ± 0.7 (0.5–2.5) | 1.4 ± 0.5 (1.0–2.1) |
| Pyruvate (n = 10) | 11,964 ± 7979 (3144–21,122) | 2.1 ± 1.5 (0.5–4.0) | 1.4 ± 0.8 (1.0–3.3) |

We are aware of only one other published OAA clinical study, which was reported in 1968 [14]. In that study the author tested the oral hypoglycemic effect of OAA in diabetic subjects and found it was well-tolerated at doses ranging from 100 to 1000 mg per day (administered in divided doses), and for durations ranging from 5 to 44 days (the 1000 mg per day dose was only tested for 5 days). Based on comparisons between pre and post-treatment fasting blood sugar levels, it was concluded that OAA did have a hypoglycemic effect. While we did not detect a similar effect, it is important to note that we studied AD subjects who did not carry a diabetes diagnosis and had lower blood sugars, the OAA doses we evaluated were lower than those tested in the majority of the diabetic subjects, and the absolute glucose readings from visit 2 were slightly lower than the visit 1 readings in 5 of our 6 subjects.

The 1968 study also reported limited PK data. Levels were not increased 30 min but were increased 60 min after 200 mg was orally administered to three subjects. On average, blood OAA levels rose from 0 ng/ml to 23 ng/ml. Clearly, both the basal and the peak OAA concentrations we report here are far higher than those related in the previous study.

The measured values from the two studies, although we would assume our LC–MS/MS approach should have greater sensitivity and specificity than the derivatization and colorimetric approach described in the earlier study.

In addition to being proposed for the treatment of AD and diabetes, recent preclinical research has also identified OAA as a potential therapeutic agent for stroke, traumatic brain injury, amyotrophic lateral sclerosis, and glioma [15–18]. The clinical safety data we now report should prove relevant to efforts intending to translate results from these preclinical studies to the clinical arena. Our study also informs our attempts to develop OAA as a treatment for AD. Overall, we conclude that although OAA 100 mg capsules twice per day for one month are safe in AD subjects, because a consistent and clear increase in the OAA plasma level was not observed future clinical studies need to evaluate higher doses.

Disclosures

The authors have no conflicts to disclose.

Transparency document

The Transparency document associated with this article can be found, in the online version

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