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Pharmacokinetic study of lipoic acid in multiple sclerosis: Comparing mice and human pharmacokinetic parameters

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Abstract

Background—Lipoic Acid (LA) is a natural anti-oxidant available as an oral supplement that different manufacturers produce. LA administered subcutaneously (SC) is an effective therapy for murine experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis (MS).

Objective—To compare serum LA levels with oral dosing in MS subjects to serum levels in mice receiving SC doses of LA.

Methods—We performed serum pharmacokinetic (PK) studies in 24 MS subjects after a single oral dose of 1200 mg LA. Subjects received one of the 3 different racemic formulations randomly: tablet (Formulation A) and capsules (Formulations B and C). Mice PK studies were performed with three different SC doses (20, 50 and 100 mg/kg racemic LA). The PK parameters included Maximum Serum Concentrations (C_{max} in μg/ml) and Area Under the $Curve_{0\text{-infinity}}$ ($AUC_{0\text{-infinity}}$ in μg*min/mL).

Results—Mean C_{max} and $AUC_{0\text{-infinity}}$ in MS subjects: group A (N = 7) 3.8 ± 2.6 and 443.1±283.9; group B (N = 8) 9.9± 4.5 and 745.2±308.7 and group C (N = 8) 10.3± 3.8 and 848.8±360.5 respectively. Mean C_{max} and $AUC_{0\text{-infinity}}$ in the mice were: 100mg/kg LA: 30.9±2.9 and 998±245; 50mg/kg LA: 7.6±1.4 and 223±20; 20mg/kg LA: 2.7±0.7 and 119±33.

Conclusions—Subjects taking 1200 mg of LA from two of the three oral formulations achieved serum C_{max} and AUC levels comparable to that observed in mice receiving 50 mg/kg SC dose of LA, which is a highly therapeutic dose in EAE. 1200 mg oral LA can achieve therapeutic serum levels in MS subjects.

Keywords

multiple sclerosis; lipoic acid; pharmacokinetics; experimental autoimmune encephalomyelitis; anti-oxidants; dietary supplements

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Introduction

Multiple sclerosis (MS) is a disabling autoimmune mediated neurologic disease that primarily affects young adults [1]. Significant advancement in the treatment options of MS has occurred in the last two decades with availability of six FDA approved disease modifying therapies (DMTs) [2]. Despite this achievement current therapies are inactive orally, only partial effective, and expensive. There therefore remains a need to find better treatment options for MS. Currently, several oral agents are being tested in phase II or III clinical trials for MS but may also suffer from safety concerns and probably will be expensive [3–7]. Availability of an effective oral DMT for MS with minimal side effects and at a reasonable cost would represent a significant breakthrough in MS therapeutics.

Lipoic acid (LA) is an oral anti-oxidant supplement that is available over the counter in the USA. LA is a pure, synthesized chemical compound and, unlike supplements isolated from biologic sources or botanical extracts, does not contain additional active biologically active ingredients. LA and its redox couple dihydrolipoic acid (DHLA) are co-factors for several mitochondrial dehydrogenases involved in ATP generation (Reviewed in [8]. While some LA is derived from the diet, LA synthase can catalyze the generation of LA in mammals. Under normal circumstances, essentially no free LA is detectable within blood. However, following oral or parenteral administration, free LA appears within blood and a variety of tissues, including the CNS [9–11]. It is converted intracellularly into its redox couple, DHLA. DHLA is a potent anti-oxidant and many of the biologic activities of LA are attributed to its ability to increase intracellular levels of DHLA (reviewed in [12]). LA also has biologic activities independent of DHLA, such as stimulating cAMP levels in CD4+ and NK cells [13,14] and these activities may relate to its ability to cross-link cysteine side-chains. LA has anti-inflammatory and neuroprotective effects in vitro and in animal models. LA has also been shown to be effective in treating diabetic polyneuropathy in humans [15–20].

LA is an effective therapy for experimental autoimmune encephalomyelitis (EAE), a widely used model of MS where it has been given subcutaneously (SC), intraperitoneally (IP) and orally [21–23]. Therapeutic effects of LA administration last for at least 45 days after immunization [21]. EAE has provided important insights into the immunopathogenesis of MS and has led to the development of new therapeutic approaches for treating MS [24,25]. Our laboratory was the first to demonstrate that LA is highly effective at suppressing and treating EAE [21], a finding confirmed by others [22,23]. LA suppresses EAE by interfering with trafficking of encephalitogenic T cells into the spinal cord. The immunomodulatory effects of LA involve several related mechanisms of action, including inhibition of MMP-9 [21,22,26] production by T cells at the mRNA level and inhibition of the expression of the adhesion molecules, ICAM-1 and VCAM-1 by CNS endothelial cells. Recently, we have demonstrated that LA stimulates cAMP production in CD4+ T cells and NK cells via the prostaglandin receptors EP2 and EP4 [13,14].

We reported the first human trial of LA in MS that related serum LA concentrations to changes in serum markers of inflammation [27]. This study compared 1200 mg and 600 mg oral doses of LA to placebo and showed 1200 mg dose to be generally well tolerated and significantly better than 600 mg in producing measurable serum LA concentrations. The study however showed significant inter-subject variability in peak serum LA concentrations. This inter-subject variability in peak serum LA concentrations may be due to high variability in absorption of LA from the gastrointestinal tract as others have reported [28,29].

It is presently unknown what constitutes a target blood concentration for LA to achieve the desired anti-inflammatory effects as we attempt to study LA as a potential therapy for MS.

The EAE experiments used subcutaneously (SC) administered LA but guided us towards a potentially therapeutic oral dose of LA in humans. In drug development, it is critical to study factors that can affect bioavailability of a therapeutic agent. LA is marketed by different manufacturers and the information about its pharmacokinetics is limited. We studied the pharmacokinetics of three different formulations of LA given orally as a single dose of 1200 mg. Our purpose was to assess the factors affecting the variability in bioavailability in MS patients and the feasibility to do a focused PK assessment in future studies of LA. We also explored whether orally administrated LA in MS subjects result in serum LA concentrations that are comparable to that achieved in mice with doses of LA that are therapeutic in EAE. This initial study will provide PK data needed to design efficacy trials of LA in MS.

Material and Methods

EAE and LA dose effect experiments

The study received approval from the Portland Veterans Affairs Medical Center Institutional Animal Care and Use Committee prior to initiation. These experiments were conducted as described in Marracci et al. [21]. Pure synthetic LA (without filler) was obtained from Tyler Laboratories, (Gresham, OR). Briefly, female SJL mice were immunized by SC injection with an emulsion containing proteolipid protein (PLP) 139–151 peptide and complete Freund's adjuvant (CFA) containing 150 μg of peptide and 200 μg of *Mycobacterium tuberculosis* in a total volume of 0.2 ml. With this method of immunization, 100% of control mice developed clinical signs of acute EAE 11–14 days after immunization and most mice have one or more spontaneous relapses of EAE after recovering from the first episode. Seven days after immunization and prior to disease onset, mice were randomized to daily SC injections of saline or racemic LA in varying doses (5, 10, 20, 50 and 100 mg/kg/day). Mice were scored for clinical EAE blinded to treatment status. The experiment was conducted as a suppression paradigm, in which mice were randomly assigned to receive 5–100 mg/kg LA or saline each day commencing on day 7 post immunization and prior to disease onset. Daily monitoring and injections continued for 45 days post immunization. The 10-Day Cumulative Disease Score (10-Day CDS) was calculated for each animal by adding the daily disease score on 10 consecutive days commencing on the first day of signs of clinical EAE in the mice receiving saline. The percentage suppression of disease for each dose of LA was calculated using the following formula:

$$\frac{(\text{Mean 10-day CDS in saline treated mice})-(\text{Mean 10-day CDS in LA treated mice})}{\text{Mean 10-day CDS in saline treated mice}}\times 100$$

Dosing and PK Sample Collection in Mice

The PK studies were performed in mice using three different doses of SC racemic LA obtained from Sigma-Aldrich. The three doses of SC LA were 100mg/kg, 50mg/kg and 20mg/kg. The PK for an individual dose was performed on a single day. We used one set of naïve and saline control mice, and collected blood at 30 minutes post injection of saline. Blood was collected by cardiac puncture at 1min, 5min, 15min, 30min, 1hr, 2hr and 5hr with three mice at each time point. Serum was recovered and stored at −80C until assay was performed.

Human Subjects

The study received approval from the Oregon Health & Science University (OHSU) Institutional Review Board prior to initiation. All subjects gave informed consent before entering the study. To qualify, subjects needed to meet the following inclusion/exclusion criteria: Definite MS by McDonald's criteria [30]; EDSS ≤ 7.5 [31]; age 18–70, inclusive;

no clinically significant MS exacerbation within 30 days of the screening; no systemically administered corticosteroids within 30 days of study entry; patient not pregnant or breast-feeding; no LA in previous 2 weeks; patient not on anti-coagulants or aspirin during the study; no significant health problems (e.g. active coronary heart disease, liver disease, pulmonary disease, diabetes mellitus) that could increase risk of patient experiencing adverse events; subject unable to give informed consent. Patients were allowed to receive symptomatic medications while on study. Subjects taking recombinant interferon (IFN)-b or glatiramer acetate were allowed to continue taking these medications.

Lipoic acid in human studies

Viatris® (now called Meda Pharma®) provided a tablet formulation containing 600 mg racemic LA (lot # 4E002-1, expiration date 05/07), referred to as Formulation A. Vital Nutrients® (Middletown, CT, USA) provided gelatin capsules containing 300 mg racemic LA (lot # 5G24 expiration 08/07). Each capsule also contained cellulose powder and small amounts of ascorbyl palmitate and silica. This product is referred to as Formulation B. Pure Encapsulations® (Sudbury, MA, USA) provided vegetable capsules containing 600 mg racemic LA (Lot # 3480504 expiration 11/06) referred to as Formulation C. Each 600 mg LA capsule contained 30 mg of ascorbyl palmitate and pine cellulose plant fiber to add to volume.

Human Study Design

This was a randomized open label trial comparing 1200 mg of three different formulations of oral LA in subjects with MS. Subjects were randomized to one of three treatment arms using 1:1 allocation with a computer-generated, blocked randomization scheme. Subjects came to the Oregon Clinical and Translational Research Institute (OCTRI) clinics at OHSU for all visits. Visit 1 consisted of a screening baseline blood draw, and neurological and physical examinations. Upon enrollment, Visit 2 occurred one week later, at which time the first dose of the study drug was administered and blood samples were collected for the pharmacokinetic study.

LA Dosing and PK Sample Collection in MS subjects

The PK studies were performed in MS subjects using a single oral 1200 mg dose of LA, using the randomly assigned formulations, Formulation A, Formulation B or Formulation C. On the day of PK study, patients had to fast from food and beverages (except for water) overnight for at least 10 hours. Patients received a standardized breakfast in the clinic. Immediately following completion of the meal, each patient received LA 1200 mg orally with 240 mls of water. Blood samples for measurement of LA concentrations in serum were obtained by venipuncture at baseline, and at 5, 10, 15, 30, 60, 90, 120, 180, 240, and 300 minutes after the dose. Blood samples were collected into untreated Vacutainer collection tubes. Serum was immediately separated from the clot by centrifugation, aliquoted (0.5 mL) and stored at −80° C until analyzed.

Lipoic acid analysis

LA and the internal standard, 2-(6-methoxynaphthalen-2-yl) propanoic acid (naprosyn), were obtained from Sigma-Aldrich (St. Louis, MO). HPLC solvents were obtained from Burdick and Jackson (Muskegon, MI) and other solvents and chemicals were from Sigma-Aldrich and were analytical grade. Strata impact protein precipitation 2 ml square filter plates, Strata 96-well collection plates, pierceable sealing mats, and 96-well plate manifold were from Phenomenex (Torrance, CA).

LA levels were determined by liquid chromatrography tandem mass spectrometry (LC/MS/MS) using an adaptation of the method of Chen et al. [32] in a 96 well plate format. Similar results were obtained using individual micro-centrifuge tubes for sample preparation. Plasma samples were thawed and a 50 μl aliquot was added to 150 μl of acetonitrile that contained 0.33 ng/μl of the internal standard, naprosyn. The samples were allowed to stand for 5 min and then vacuum filtered into the collection plate. A 5 μl sample of the acetonitrile extract was used for analysis. If individual micro-centrifuge tubes were used, the samples were vortexed and the protein precipitate removed as described below for saliva samples. For all patients a baseline plasma sample (i.e. time = 0) was prepared in the absence of naprosyn and spiked with 1000 ng/ml of LA. If the samples contained naprosyn, the amount of LA was calculated from the area of LA alone corrected for the recovery from the spiked sample.

Samples were analyzed using a Thermo TSQ Quantum Discovery triple-quadrupole mass spectrometer (San Jose, CA) equipped with an electrospray ionization source. All mass analyzers were operated at unit mass resolution. The ionization interface was operated in the negative mode using the following settings: spray voltage, 2,000 V; sheath gas flow rate, 25 ml/min; tube lens offset, 35 V; and capillary temperature, 275 °C. LA and naprosyn were monitored by selective reaction monitoring (SRM) with a collision energy of 8 monitoring the transitions of m/*z* 205.0→171.00 for LA and *m*/*z* 229.0→185.0 for naprosyn.

The LC-MS system was composed of an in-line Acella auto-sampler and HPLC pump (ThermoFisher, San Jose, CA). LA and naprosyn were resolved from other plasma components using a 2.1 × 50 mm, 3.5 μm Zorbax SB-C18 and a 5 μm 2.1 × 12.5 mm guard column (Agilent Technology, Wilmington, DE) maintained at 30 °C. The HPLC mobile phase consisted of water with 0.03% acetic acid (solvent A) and methanol with 0.03% acetic acid (solvent B) delivered at a flow rate of 0.3 ml/min. The column was equilibrated with 30% solvent B and then increased to 95% solvent B in 3.0 min, held for 1.5 min and returned to 30% solvent B and equilibrated for 2.4 min. The injection volume was 5 μl. LA and naprosyn eluted at 3.09 and 3.31 min, respectively. A series of standards from 5 to 50000 ng/ml LA were prepared in naïve serum with each sample set. A methanol stock of LA stored at −20 °C was diluted in water to prepare working dilutions on the day of use. Linear least-square regression of the plasma concentrations and measured peak area ratios using a 1/x weighting was used for the quantification. The intra-day coefficient of variation at 5 and 50,000 ng/ml was 8.5% and 3.4%, respectively. The inter-day coefficient of variation was 7%. Data acquisition and quantitative processing were accomplished with Xcalibur software.

Statistical Analyses

Serum LA concentration-time data were analyzed by non-compartmental methods using the WinNonLin software program Version 4.1 (Pharsight Corporation, Mountain View, CA). Maximum serum LA concentration (C_{max}) and time to occurrence (T_{max}) were the observed values. Area under the serum concentration-time curve from time zero until the last concentration-time point ($AUC_{0\text{-}last}$) was calculated by the linear trapezoidal rule. Terminal area was the quotient of the last concentration divided by the terminal elimination rate constant (λ_z). This constant was determined from regression analysis of concentration-time points in the terminal elimination phase. AUC from time zero to infinity ($AUC_{0\text{-}\infty}$) was the sum of $AUC_{0\text{-}last}$ plus terminal area. Terminal elimination half-life ($t_{1/2}$) was calculated as $0.693/\lambda_z$, oral clearance (CL/F) was calculated as oral dose/$AUC_{0\text{-}\infty}$, and volume of distribution (V) was calculated as CL//λ_z.

Data were normally distributed and were reported as mean ± S.D. Pearson's product correlation coefficients were calculated to determine the linear association between patient

age, height, weight, and body mass index and LA PK parameters ($t_{1/2}$, V, CL/F, and $AUC_{0\text{-last}}$). Multiple group comparisons (e.g. Formulation A vs. Formulation B vs. Formulation C) were done using analysis of variance (ANOVA) with Tukey's post test. Statistical significance was defined as $P < 0.05$. SPSS for Windows Version 15.0 (SPSS Inc., Chicago, IL) was used for the statistical analyses.

Results

Relationship between LA PK and suppression of EAE

EAE and LA dose response experiments were conducted, where LA was administered to the mice at doses of 5, 10, 20, 50 and 100 mg/kg/day beginning on day 7 after immunization. Figure 1 shows a clear dose response of LA as assessed by the percentage suppression of the 10-day CDS in the EAE mice at the various doses of LA. The percentage suppression of the 10-day CDS in mice treated with the three doses of LA that were used in the PK study, 20, 50 and 100 mg/kg/day were 54±13, 91±14, and 98±5 %, respectively.

Figure 2 and Table 1 show the PK profiles and PK/PD parameters of three different doses (20, 50, and 100 mg/kg) of LA in mice. The serum C_{max} (μg/ml) values for 20, 50, and 100 mg/kg doses of LA were 2.7±0.7, 7.6±1.4, and 30.9±2.9 respectively. The corresponding $AUC_{(0\text{-}60min)}$ values for these doses were 44±3.3, 147±25, and 781±247 μg*min/ml respectively. We thus find that C_{max} and AUC of LA are positively related to the percentage suppression of 10-day CDS scores in mice.

LA PK results in MS subjects

For the human PK study, we screened 36 subjects with MS. Out of the 36 subjects 29 were randomized to participate in the study. Five of the subjects after randomization withdrew. Reasons for withdrawal are described in Figure 3. Twenty-four subjects completed the study. Serum data was available for analysis for 23 subjects as there was contamination in one subject's serum samples and was not analyzable. All subjects tolerated the study drug post administration. Subject demographics are outlined in Table 2.

Table 3 shows the LA PK parameters for the three formulations after a single 1200 mg oral dose. The C_{max} for Formulations A, B and C were 3.81±2.66, 9.98±4.53 and 10.28±3.82 μg/mL, respectively. Figure 4 shows the serum LA concentration-time profiles for Formulations A, B and C. Wide inter-patient variability was observed at each concentration-time point. However, C_{max} was found to be significantly lower in patients receiving Formulation A compared to the other formulations ($P < 0.05$).

The highest AUC was found in subjects receiving Formulation C while the lowest AUC was found in subjects receiving Formulation A (AUC = 821±327 vs. 392±290 μg*min/mL; $P = 0.037$). Accordingly, a trend towards a faster oral clearance was found in patients receiving Formulation A compared to Formulation C and Formulation B (CL/F = 3849.9 ± 2800.6 mL/min vs. 1804.9 ± 1207.5 and 1860.5 ± 740.1 mL/min; $P = 0.060$). Volume of distribution was significantly higher in patients receiving Formulation A than the other formulations.

Linear associations between LA PK parameters vs. weight and body mass index (BMI) were calculated for the three LA products. Moderate to strong correlations were found between CL/F, V_D and AUC and weight and BMI ($P < 0.05$) in patients receiving Formulation B (data not shown) and Formulation C. As body weight and BMI increased, CL/F and V_D increased while AUC decreased. Figures 5a–5h show the linear associations between different PK parameters vs. weight and body mass index (BMI) for formulation C. There was no significant linear association between the $t_{½}$, CL/F, V_D and AUC of LA vs. weight

or BMI with formulation A. LA PK parameters were not significantly correlated with age or height (data not shown).

Discussion

This is the first study relating serum LA levels in humans to therapeutic levels in a mouse model of a human disease. We demonstrated that a 1200 mg dose of LA given orally to MS subjects produces C_{max} levels comparable to that of mice receiving 50 mg/kg, which is highly effective at suppressing EAE. LA has been used in clinical trials in diabetic polyneuropathy, Alzheimer's disease, peripheral arterial disease, migraine and burning mouth syndrome. However, doses chosen for these trials were not based on target serum levels of LA [15–20, 33–42]. In the diabetic neuropathy trials the dose of LA varied between different trials (oral dose varied from 100–1800 mg/day and IV dose varied from 600 – 1200 mg/day). In non diabetic neuropathy trials, the dose of LA ranged from 200 mg to 800 mg/day [35–42]. Some of the clinical trials where LA was not shown to be beneficial may have been limited by sub optimal dosing of LA resulting from a failure to do PK studies. In particular trials using oral LA less than 600 mg may have used sub therapeutic dose because our previous results have indicated that 600 mg does not produce detectable levels in many subjects at least in the age group that we have studies [27]. We thus believe that it is important to measure serum levels of LA in any future clinical trial. Based on the results obtained in this study, we now know that 1200 mg of LA provides a blood level that is therapeutic in a relapsing mouse model of MS.

In this study we also compared the pharmacokinetics of 1200 mg oral LA across three different formulations. While supplement companies often recommend taking LA before eating, we administered all three formulations of LA orally with food to minimize gastric side effects. Similar to previous reports, we found high inter-subject variability in pharmacokinetics of LA [27, 28]. Ingestion of LA with food may have contributed to the variability. Also, our use of LA in tablets vs. capsules (Formulations A vs. B and C) and 4 capsules vs. 2 capsules (Formulation B vs. C), may have added to the variability. Importantly, all three formulations gave detectable serum levels of LA in all subjects.

Significant differences in the PK parameters between the three formulations were evident by this study. Formulations B and C were comparable in several of the PK parameters and achieved easily detectable LA levels (C_{max} = 9.99 μg/ml and 10.3 μg/ml respectively). Formulation A exhibited lower C_{max} and AUC than the other two formulations. One potential explanation for lower C_{max} and AUC with Formulation A, is our non-conformity to the manufacturer's recommended administration without food.

This study also explored various factors affecting the variability in the PK parameters of LA and found a correlation between body weight and LA PK parameters, CL and AUC. While CL increased with body weight, AUC decreased with body weight. It is well established that renal clearance increases with body weight [43, 44]. This suggests that LA may be significantly cleared by renal route. However, other studies conducted with 600 mg dose have shown that the pharmacokinetics of LA are not influenced by creatinine clearance, an indicator of renal function [45]. Further, renal excretion of LA was shown to account for <10% of parent drug [46] in contrast to >80% of oral dose in urine in animals [47]. We speculate that in the absence of significant renal excretion, non-renal metabolism of LA might have increased with body weight. However, we do not have conclusive evidence to support this speculation. On the other hand, the current study used a higher dose (1200 mg vs. 600 mg) and it is possible that at higher doses protein binding and/or non-renal metabolism of LA might be saturated and therefore renal excretion becomes significant.

This speculation is untested and warrants a closer examination as this may be of importance when using high doses LA in patients with compromised renal function.

LA appears to work through a novel mechanism of stimulating cAMP via the EP2 and EP4 receptors which in turn activates protein kinase A (PKA) resulting in the transcription of a number of different genes[13,14,48]. A once a day pulse of LA therefore, should be effective given the cascade of events that ensue from PKA activation [49]. This is consistent with our findings in EAE in which once a day dosing is highly effective. Based on the apparent mechanism of action, our EAE studies and our PK studies, we believe that oral administration of 1200 mg once a day of LA in MS subjects would be the appropriate regimen to test in a clinical trial.

Conclusions

Subjects taking 1200 mg of LA from two of three formulations achieved serum C_{max} and AUC levels comparable to that observed in mice receiving 50 mg/kg dose of LA, which is a highly therapeutic dose in EAE. Orally administered LA at a dose of 1200 mg can achieve therapeutic serum levels in MS subjects.

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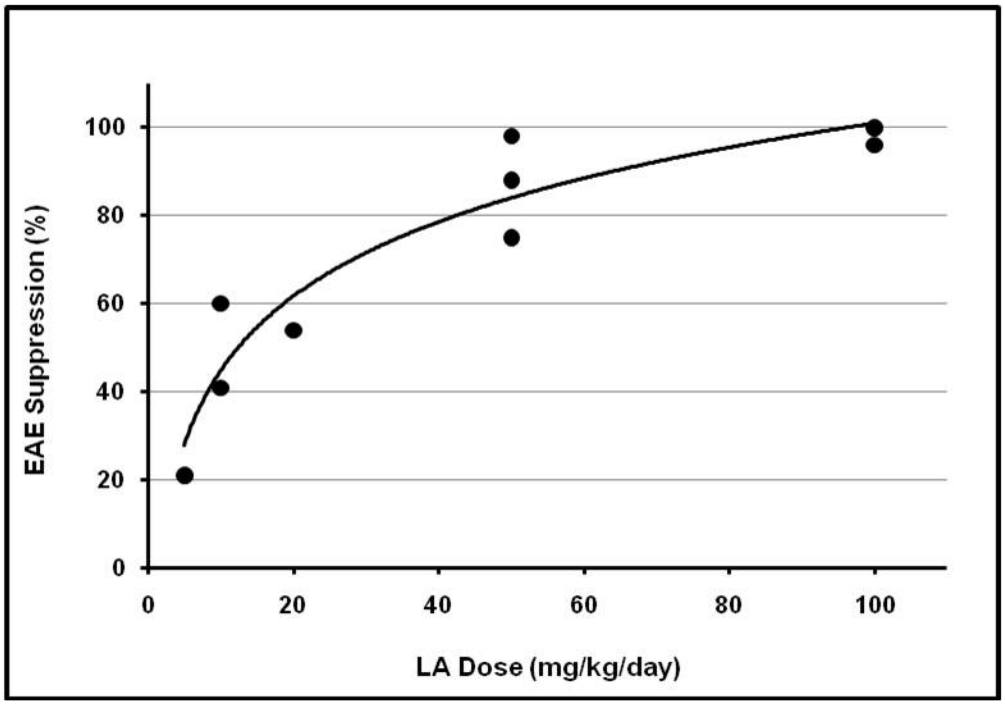

Figure 1. LA suppresses EAE: Dose Response Curve

EAE was induced in SJL mice and percentage suppression was calculated after LA was administered beginning at day 7 post immunization as is described in the methods section. The graph represents EAE suppression vs. dose of LA. The 5 mg/kg group (n=8) exhibited a mean of 26.1 +/− 10.5, with 23% EAE suppression. The two 10 mg/kg groups (each n=5) showed means of 11.4 +/− 7.3 with 60% EAE suppression and 11.2 +/− 3.3 with 42% suppression. The 20 mg/kg group (n=8) exhibited a mean of 15.6 +/− 10.7 with 54% EAE suppression. The three 50 mg/kg groups (n=5, n=8 and n=5) demonstrated means of 4.6 +/− 6.2 with 76% suppression, 4.1 +/− 5.5 with 88% suppression and 0.4 +/− 0.9 with 99% suppression, respectively. The two 100 mg/kg groups (each n=5) exhibited means of 1.2 +/− 2.6 with EAE suppression of 94%, and 0 +/− 0 with suppression of 100%.

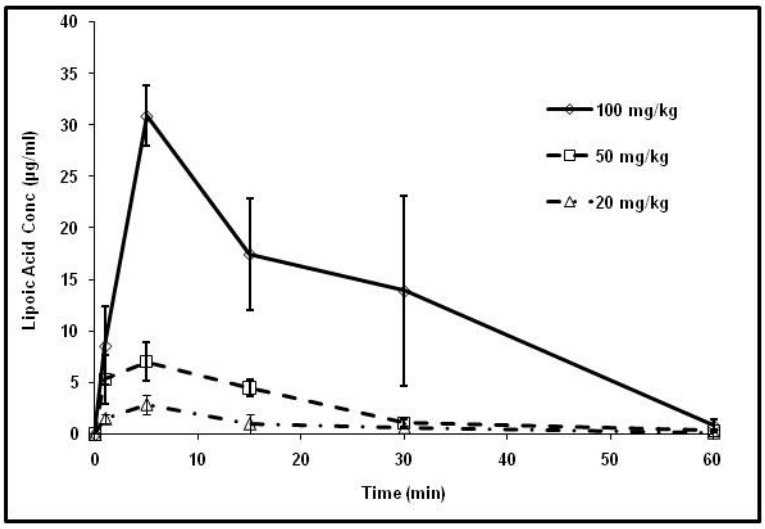

Figure 2. Serum lipoic acid concentration-time profiles of various doses in mice

20 mg/kg, 50 mg/kg and 100 mg/kg LA was given to mice and Cmax in the sera of mice were determined. N=3 for all trials within each dose, with the error bar denoting a standard deviation of 1.

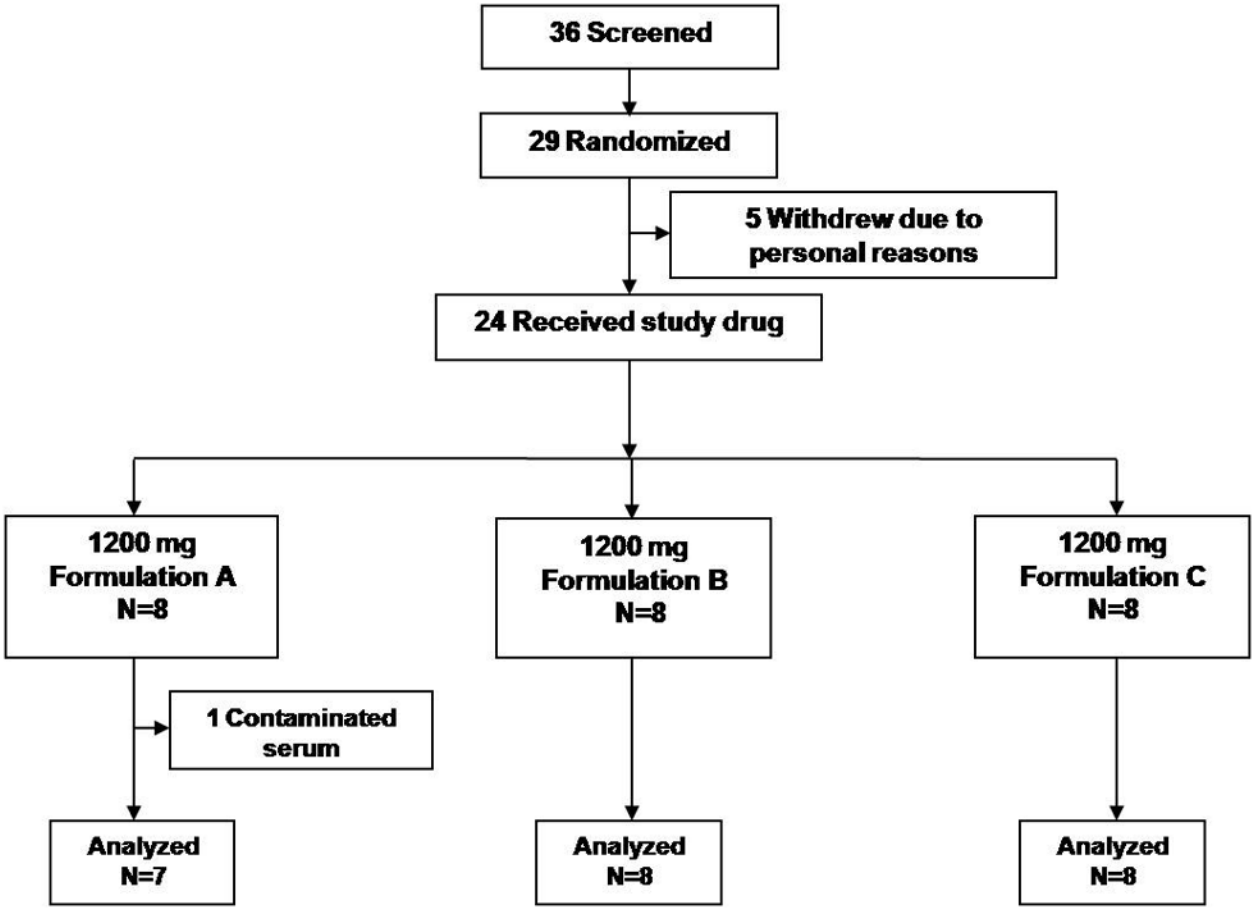

Figure 3. LA trial subject disposition

N denotes the number of subjects in each treatment group.

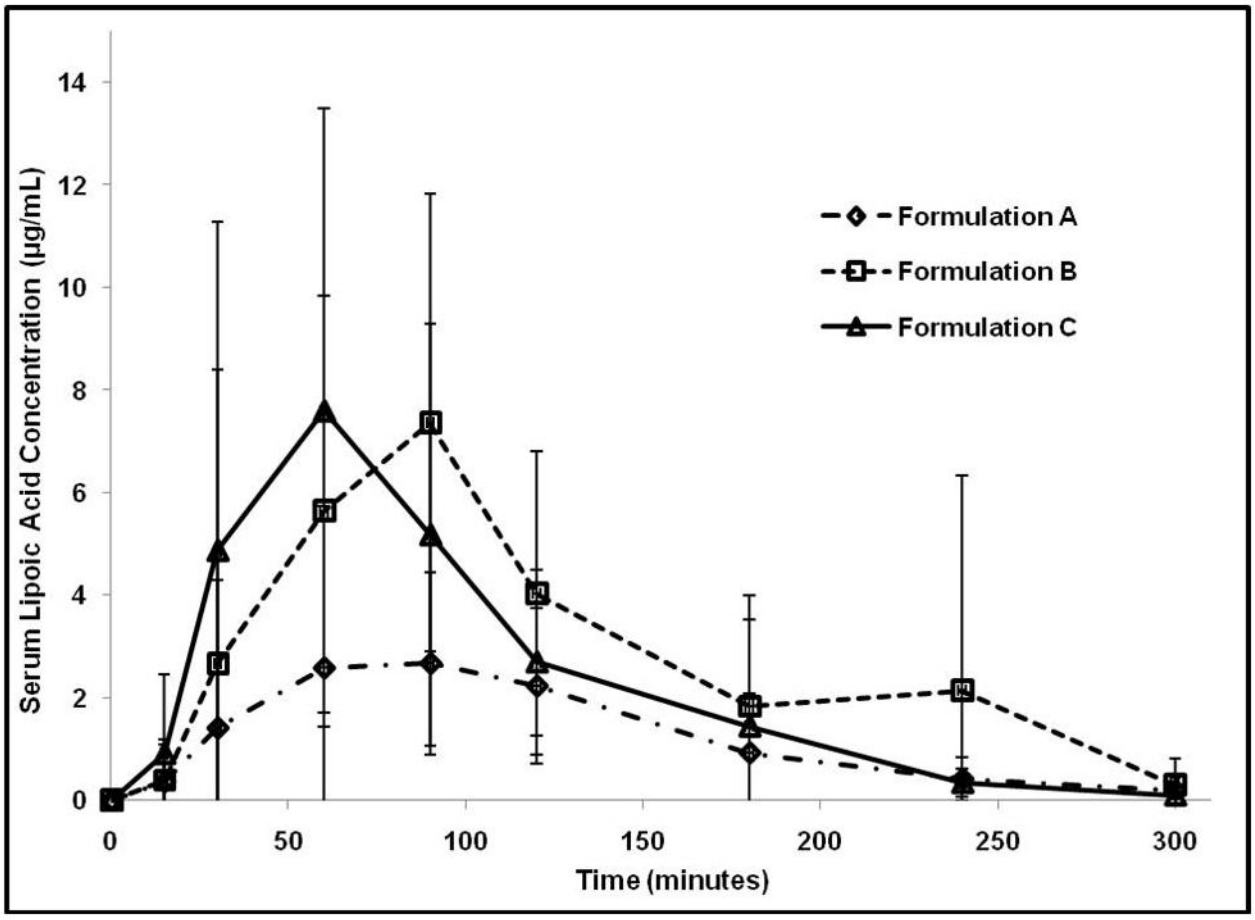

Figure 4. Serum lipoic acid concentration-time profiles for each formulation

Each data point represents the mean serum LA concentration at each time point. (Formulation A, n=7; Formulation B, n=8 and Formulation C, n=8). The error bars denote a standard deviation of 1.

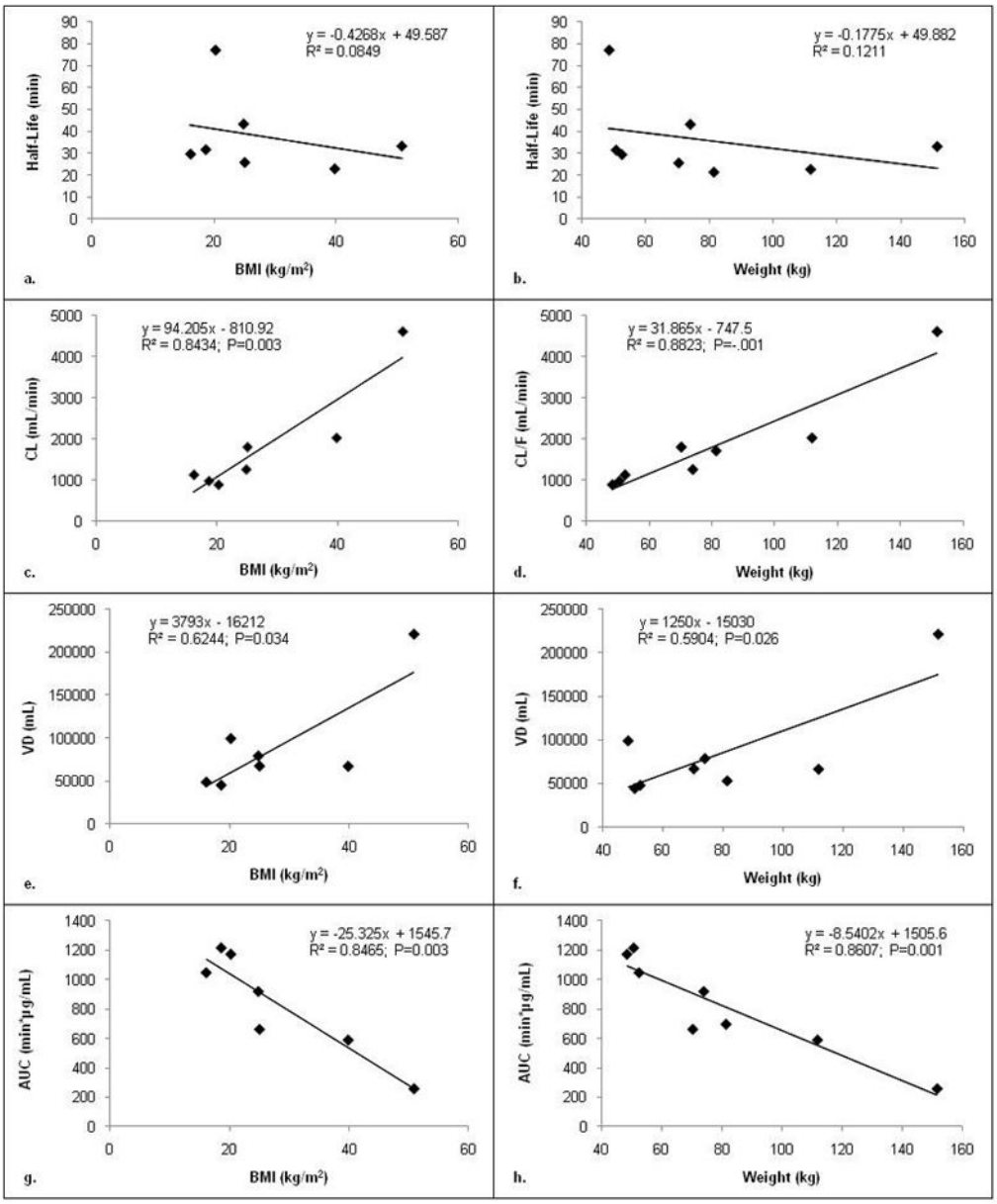

Figure 5. Linear associations between lipoic acid pharmacokinetic parameters versus body weight and body mass index for Formulation C

Panels 5(a)–5(h) represent the correlation between body mass index (BMI) and weight of subjects to the following: half life of serum lipoic acid (LA) (5(a) and 5(b)); clearance (CL/F) of oral LA (5(c) and 5(d)); volume of distribution (VD) of oral LA (5(e)and 5(f)); area under the curve (AUC) of oral LA (5(g) and 5(h)).

Table 1

Pharmacokinetic and pharmacodynamic parameters of lipoic acid in mice:

PK/PD parameter	Dose of lipoic acid		
	100mg/kg	50mg/kg	20mg/kg
*C_{max} (μg/ml)	30.9±2.9	7.6±1.4	2.7±0.7
*AUC $_{(0\text{-}60min)}$ (μg*min/ml)	781±247	147±25	44±3.3
*Suppression of the 10-day CDS (%)	98±5	91±14	54±31

C_{max} and AUC of LA respectively are inversely related to the 10-day CDS in mice.

*Denotes the mean and standard deviation of change of the values

Table 2

Baseline subject demographics:

	Formulation A N=8*	Formulation B N=8	Formulation C N=8	Total N=24
Gender, M/F	3/5	3/5	1/7	7/17
Age (years)				
Median	48.5	52	50.5	51
Range	36–66	27–77	42–65	27–77
Race				
Caucasian	8	8	8	24
Non-Caucasian	0	0	0	0
Type of MS				
Relapsing-remitting	4	3	5	12
Primary progressive	1	0	0	1
Secondary progressive	3	5	3	11
MS duration median (range in yrs)	11.5 (1–16)	21 (8–33)	11.5 (2–35)	12.5 (1–35)
EDSS median (range)	4.0 (2.0–7.0)	5.0 (2.5–7.5)	4.0 (2.0–8.0)	4.0 (2.0–8.0)

*N denotes the number of subjects in each treatment group.

Table 3

Lipoic acid pharmacokinetic parameters after a 1200 mg oral dose (mean ± SD):

PK parameters	Formulation A N = 7*	Formulation B N = 8	Formulation C N = 8
Half-life (min)	81.2 ± 97.1	40.1 ± 17.1	35.7 ± 18.2
T_{max} (min)	94.3 ± 50.3	71.3 ± 47.9	78.8 ± 27.5
C_{max} (μg/mL)[a]	3.81 ± 2.66	9.98 ± 4.53	10.28 ± 3.82
C_{last} (μg/mL)	0.21 ± 0.24	0.11 ± 0.05	0.30 ± 0.53
$AUC_{0\text{-last}}$ (μg*min/mL)[b]	392 ± 290	739 ± 308	821 ± 327
$AUC_{0\text{-infinity}}$ (μg*min/mL)	443 ± 283	745± 308	848± 360
Volume of Distribution (mL)[c]	377083.7 ± 359686.8	107316.1 ± 58474.8	85093.2 ± 57905.6
Oral Clearance (mL/min)	3849.9 ± 2800.6	1860.5 ± 740.1	1804.9 ± 1207.5

*Number of subjects in each group (N)

[a]Formulation A vs. Formulation B $P = 0.013$; Formulation A vs. Formulation C $P = 0.010$

[b]Formulation A vs. Formulation C $P = 0.037$

[c]Formulation A vs. Formulation B $P = 0.046$; Formulation A vs. Formulation C $P = 0.030$