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Lipoic acid in multiple sclerosis: a pilot study

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Lipoic acid (LA) is an antioxidant that suppresses and treats an animal model of multiple sclerosis (MS), experimental autoimmune encephalomyelitis. The purpose of this study was to determine the pharmacokinetics (PK), tolerability and effects on matrix metalloproteinase-9 (MMP-9) and soluble intercellular adhesion molecule-1 (sICAM-1) of oral LA in patients with MS. Thirty-seven MS subjects were randomly assigned to one of four groups: placebo, LA 600 mg twice a day, LA 1200 mg once a day and LA 1200 mg twice a day. Subjects took study capsules for 14 days. We found that subjects taking 1200 mg LA had substantially higher peak serum LA levels than those taking 600 mg and that peak levels varied considerably among subjects. We also found a significant negative correlation between peak serum LA levels and mean changes in serum MMP-9 levels ($\tau = -0.263$, $P = 0.04$). There was a significant dose response relationship between LA and mean change in serum sICAM-1 levels ($P = 0.03$). We conclude that oral LA is generally well tolerated and appears capable of reducing serum MMP-9 and sICAM-1 levels. LA may prove useful in treating MS by inhibiting MMP-9 activity and interfering with T-cell migration into the CNS.
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Introduction

Migration of T cells into the CNS is critical to the development of new inflammatory lesions in multiple sclerosis (MS).[1–3] Entry of autoreactive T cells across the blood–brain barrier is facilitated by tight binding between integrins and their counter-ligands, such as intercellular adhesion molecule-1 (ICAM-1) on endothelial cells and leukocyte function-associated antigen (LFA)-1 on T cells.[4] Activated T cells produce proteases, such as matrix metalloproteinase-9 (MMP-9) that facilitate cellular migration into the CNS by degrading components of the extracellular matrix.[5–9] Increased serum levels of sICAM are seen in a number of inflammatory[10–13] and atherosclerotic[14–16] diseases where it appears to reflect endothelial cell activation. In MS, increased serum levels of sICAM-1 have been associated with inflammatory disease activity and with the appearance of gadolinium-enhancing lesions on brain magnetic resonance imaging (MRI).[17–19] Similarly, previous studies have correlated changes in serum MMP-9 activity with relapses of MS.[20] Therapeutic agents that target MMP-9 and adhesion molecules may prove useful as treatments for MS and other neurologic diseases in which migration of inflammatory cells plays a pathogenic role.

Lipoic acid (LA) is an antioxidant and dietary supplement that suppresses and treats the animal model of MS, experimental autoimmune encephalomyelitis (EAE).[21,22] In EAE, LA inhibits the ability of T cells to migrate into the CNS and appears to do so in part by inhibiting the activity of MMP-9.[21–23] Trials in humans with diabetic polyneuropathy have demonstrated that LA is well tolerated and is beneficial in controlling dysesthesias.[24–26] In this study we sought to determine the tolerability and peak serum levels of LA following oral administration in MS patients and assess its effects on serum MMP-9 and sICAM-1.

Materials and methods

Subjects

This 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 diagnosis by McDonald criteria;[27] Expanded Disability Status Scale (EDSS) of <7.5;[28] age 18–70; no relapses within 30 days of screening; no LA in two weeks preceding screening; not pregnant or breastfeeding and using an acceptable form of birth control. Subjects taking

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recombinant interferon (IFN)-β or glatiramer acetate were allowed to continue taking these medications.

Study design

This was a randomized double-blind parallel treatment trial comparing three doses of oral LA with placebo. Subjects were randomized to one of four treatment arms using 1:1 allocation with a computer-generated, blocked randomization scheme: placebo twice a day; LA 600 mg twice a day (LA 600 mg BID); LA 1200 mg in morning and placebo in evening (LA 1200 mg QD); or LA 1200 mg twice a day (LA 1200 mg BID). Subjects came to the OHSU General Clinical Research Center 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. Visit 3 comprised blood collection 24 hours after the first dose of the study drug. Visit 4 followed one week and Visit 5 two weeks from the start of the study drug.

LA and placebo

Pure Encapsulations (Sadbury, MA, USA) provided gelatin capsules containing 200 mg (for 600 mg twice a day group) and 400 mg (for the 1200 mg groups) of LA and encapsulated placebo. The placebo capsules contained Avicel™ (microcellulose crystal) and 4.3 mg quercetin (a bioflavonoid) to provide a yellow coloration similar to that of LA. A pharmacist at OHSU prepared and labelled bottles containing the appropriate study drug. All subjects took three capsules twice a day before meals, for two weeks. Quantification of LA content in the capsules was independently verified by HPLC at OHSU. Capsules for LA quantification were randomly selected from the bulk batches of study drugs as supplied by the manufacturer. The mean quantity of LA in six of the 200 mg capsules was 224 mg (range 203–244) and the mean in six of the 400 mg capsules was 442 mg (range of 416–505). No LA was detected in any of six placebo capsules.

Pharmacokinetics

Blood samples were collected by venipuncture into untreated Vacutainer® collection tubes. Serum was immediately separated from the clot by centrifugation, aliquoted (0.5 mL) and stored at -80°C until analysed. Sera for LA determination were prepared at the following time points: immediately before taking the first dose of the study drug, and at 15, 30, 60, 120, 180 and 240 minutes (Visit 2) and 24 hours after the first dose of the study drug (Visit 3).

LA determination

LA levels in sera and capsules were quantified by high performance liquid chromatography (HPLC) with electrochemical detection using a modification of a procedure described by Sen and colleagues.[29] Contents of the capsules containing placebo, 200 mg of LA and 400 mg LA were weighed, dissolved in 0.1 M perchloric acid (PCA), and assayed for LA by HPLC. Analyses were performed blinded to the contents of the capsules and treatment status of the sera. All capsule quantitation and analyses of sera samples from an individual were performed during a single session by HPLC. Samples were deproteinized by combining equal volumes of serum sample and 0.2 M PCA, vortexing and placing the mixture on ice for 60–90 minutes, and then removing the precipitate by centrifugation at 14 000 rpm for 5 minutes. The LA in the supernatant was stable under these conditions for at least 24 hours at room temperature and for greater than one month at -80°C. Chromatographic analysis of LA was performed by reversed-phase HPLC with electrochemical detection on a C18 column (15×0.46 cm, 5 μm particle size; Rainin) using a mobile phase consisting of 25 mM sodium phosphate (pH 2.7), 20% acetonitrile and 20% methanol. An ESA Coulchem II multielectrode detector was used to quantify LA, with electrode 1 set at $+0.40$ V and electrode 2 set at $+0.85$ V. Electrode 1 oxidizes interfering substances in the sample matrix, and the oxidation signal for LA appears in electrode 2. Standards were prepared by addition of authentic LA (2.6 μg/mL, Sigma) to serum obtained from volunteers that contained no detectable LA signal. Using a detector sensitivity of 200 or 500 nA, we were able to accurately and reproducibly detect LA levels as low as 0.01 μg/mL in the serum.

Serum MMP-9, tissue inhibition of metalloproteinase-1 (TIMP-1) and sICAM-1

Serum was prepared as described above. MMP-9, TIMP-1 and sICAM-1 ELISA kits were obtained from R&D Systems (Minneapolis, MN, USA cat #s DMP900, DTM100 and BBE1B, respectively). Briefly, these ELISA assays rely on the standard quantitative sandwich enzyme immunoassay technique. Each 96-well plate is precoated with a monoclonal antibody specific for MMP-9, TIMP-1 or sICAM-1 proteins. Relevant standards supplied with each kit were reconstituted in deionized water and a dilution series was generated per manufacturer's instructions. Serum samples were diluted 1:100 and added to triplicate wells followed by a 1.5–2-hour incubation with horseradish peroxidase (HRP) conjugated detection antibody at room temperature with gentle mixing (50 rpm). Subsequent incubations and washes were conducted using kit reagents per manufacturer's instructions. Thirty minutes following the final incubation in HRP-substrate, the reaction was stopped and the plate was immediately analysed in a Molecular Devices Kinetic Microplate Reader at 450 nm using SOFTmax software (version 2.35) and the concentration of each target protein was derived from the standard curve.

Clinical and safety monitoring

Safety and tolerability were evaluated throughout the study by reviewing patient's self-reporting and results of specific tests. Subjects had chemistry panel, complete blood counts and urine analysis performed before and seven and 14 days after starting the study drug. Neurologic and physical examination, EDSS, 25-foot timed walk and

9-hole peg test were performed before and 14 days after starting the study drug.

Statistical analyses

Statistical analyses were done in SAS® v. 8.01 (SAS Institute Inc., Cary, NC). Differences in the baseline characteristics for categorical variables were evaluated using Pearson's chi square test or Fisher's exact test when appropriate. The Kruskall–Wallis test was used for continuous variables to evaluate differences among the groups followed by Dwass, Steel, Critchlow–Flinger all-treatments pair-wise multiple comparison test based on pair-wise rankings using Bonferroni adjusted critical values. The Jonckheere–Terpstra test was carried out for *a priori* established ordered alternatives and Kendall's tau correlation coefficient was computed. The associated slope and intercept estimators were calculated using nonparametric simple linear regression to test for correlation between continuous variables. Data were analysed only for those patients who started the study drug.

Results

Trial profile

Thirty-seven subjects met entry criteria and were randomized as described (Figure 1). Thirty subjects completed the protocol and seven discontinued. Four subjects withdrew before taking the first dose. Among them, two subjects had difficulty with venipuncture, one subject had a relapse and one subject withdrew for personal reasons. Thirty-three subjects started the study drug and three withdrew after the drug was initiated. The reasons for withdrawal included early termination due to an adverse event in one subject in the 1200 mg BID group and personal reasons for two subjects in the placebo group who refused to participate after Visit 2.

Demographics and baseline characteristics of all 33 subjects who took at least one dose of the study drug are presented in Table 1. No significant differences existed among the four study groups in age, gender, ethnicity, education level, type or duration of MS and MS-related disability as measured by EDSS, timed walk and 9-hole peg test.

LA pharmacokinetics (PK)

PK data were analysed for seven placebo, seven LA 600 mg BID, nine LA 1200 mg QD and eight LA 1200 mg BID subjects. Subjects in the 1200 mg QD and 1200 mg BID groups were considered together as their blood was collected for PK measurements after the initial 1200 mg dose. Peak LA levels correlated with dose and varied among subjects. LA was not detected in any serum samples from subjects prior to taking LA. The placebo group had negligible levels (all values <1 μg/mL) of LA with peak levels ranging between 0 and 0.8 μg/mL (median 0.1 μg/mL). Median values of peak LA serum levels for subjects taking 1200 mg were substantially higher than for subjects taking 600 mg (4.8 μg/mL versus 0.2 μg/mL, $P<0.05$) or placebo (0.1 μg/mL, $P<0.05$) (Figure 2). Considerable intersubject variability was observed both in the peak LA level and in the time to obtain peak levels (Figure 2). For subjects taking 600 mg, the peak levels ranged between 0 and 3.7 μg/mL, with time to peak ranging from 60 to 240 minutes (median 180 minutes). Subjects taking 1200 mg developed peak levels that ranged between 0 and 19 μg/mL with time to peak ranging from 60 to 240 minutes (median 120 minutes). LA was not detectable in serum samples taken 24 hours after the initial dose.

Safety and tolerability

Table 2 shows the adverse events among the different groups. None of the reported side effects reached a statistically significant level. Three subjects needed

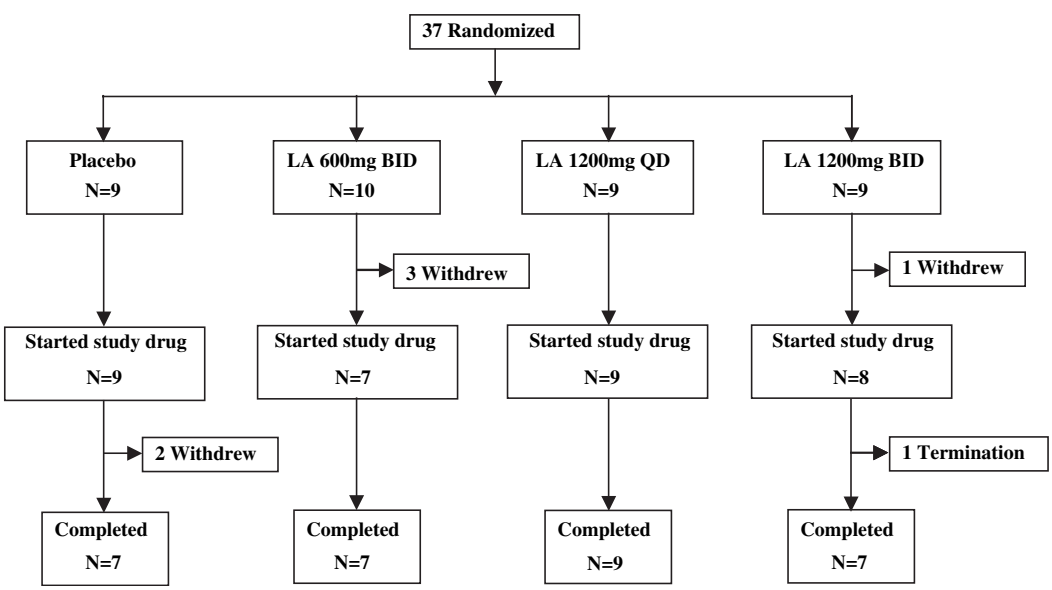

Figure 1 *LA trial subject disposition.* n *denotes the number of subjects in each treatment group.*

Table 1 Baseline demographics and clinical characteristics

Characteristics	*Placebo*	*LA*			*Total*
		600 mg BID[a]	*1200 mg QD*[b]	*1200 mg BID*	
Number in group (*n*)	9	7	9	8	33
Gender, M/F	2/7	1/6	1/8	0/8	4/29
Age (years)					
Median	50	49	54	44.5	49
Range	36–66	39–60	37–67	34–56	34–67
Race					
Caucasian	8	6	9	7	30
Non-Caucasian	1	1	0	1	3
Type of MS					
Relapsing–remitting	2	3	8	4	17
Primary progressive	3	2	0	1	6
Secondary progressive	3	2	1	2	8
Unclear	1	0	0	1	2
MS duration in years, median (range)	14 (4–35)	18 (1–25)	14 (4–27)	16 (1–35)	17 (3–30)
EDSS median (range)	4 (2.5–6.5)	4 (1.5–6.5)	4 (1.5–6.5)	4 (0–6.5)	4 (0–6.5)

[a]BID, twice a day; [b]QD, once a day.

adjustment of the study drug due to nausea (one in LA 600 mg BID group and two in LA 1200 mg QD group). One subject taking LA 1200 mg BID developed a disseminated maculopapular allergic rash with fever on day eight. In this patient mild thrombocytopenia was noted at the time of rash. The study drug was stopped and the rash and thrombocytopenia resolved without treatment. The most common side effects were malodorous urine and gastrointestinal side effects such as mild nausea and discomfort; these side effects are similar to those observed by Ziegler *et al*. in trials of LA for diabetic polyneuropathy.[30] No significant change was observed in any of the laboratory parameters evaluated including complete blood count, metabolic panel or urine analysis in all other patients.

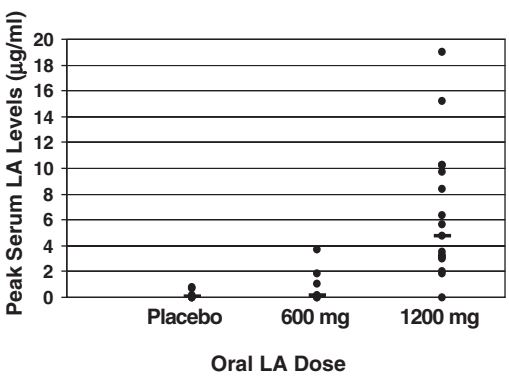

Figure 2 *Peak serum LA levels following the first dose of the study drug. Each dot represents a single subject. The horizontal bar represents the median LA level for the three groups. The graph shows wide variability in peak levels in both 600 mg and 1200 mg groups. The placebo group had negligible levels (all values <1 µg/mL) of LA. Median peak LA serum level for those taking 600 mg was 0.2 µg/mL (range 0–3.7 µg/mL) and 1200 mg was 4.8 µg/mL (range 0–19 µg/mL).*

All subjects remained clinically stable during the two-week study.

LA and MMP-9 levels

The baseline MMP-9 levels were drawn on Visit 2 prior to patients getting the study drug. All patients had data available on their MMP-9 and TIMP-1 levels at baseline and on Visit 4. MMP-9 and TIMP-1 data were not available for two patients on their third visit and five for their fifth visit as they missed the blood draws. The mean change in MMP-9 and TIMP-1 levels compared with baseline for Visits 3, 4 and 5 were used as the outcome measure. There were no significant differences between the four groups in baseline MMP-9 and TIMP-1 levels. There were no significant differences for the median change in MMP-9 or TIMP-1 among the four groups and no significant dose response relationship was found for either MMP-9 ($P = 0.39$) or TIMP-1 ($P = 0.45$). Because of the wide variability in peak serum LA levels, we investigated the relationship between peak LA levels and MMP-9 and TIMP-1 levels in the serum. There was a significant negative correlation between the peak serum LA values and the MMP-9 mean change (Kendall's $\tau = -0.263$, $P < 0.04$) (Figure 3). A change in the peak LA level of 1 µg/mL led to a change in mean MMP-9 from baseline of -11.10 units (95% CI -35.00, -0.71). There was no significant correlation between the peak LA values and mean TIMP-1 change (Kendall's $\tau = 0.049$, $P > 0.5$).

LA and sICAM-1 levels

No significant differences were revealed in baseline values for sICAM-1 among the four study groups. The mean change of Visits 3, 4 and 5 as compared with baseline sICAM-1 was used as the outcome measure. The three study groups that received LA had a decrease in their sICAM-1 levels for all three visits though the difference among the study groups was not statistically significant.

Table 2 Adverse events

Side effects	*Placebo* (n[a] = 9)	*LA 600 mg BID* (n = 7)	*LA 1200 mg QD* (n = 9)	*LA 1200 mg BID* (n = 8)	P
Urine smell	0	0	3	1	0.14
Nausea/upset stomach	1	1	4	4	0.20
Generalized weakness	0	0	1	2	0.37
Rash and fever	0	0	0	1	0.45
Headache	0	0	2	1	0.47
Pain/spasms	0	0	1	1	0.84

[a]*n*, the number of subjects in each treatment group.

However, a significant dose response relationship was observed for the mean change in sICAM-1 with those taking the higher doses having the greatest decrease in sICAM-1 (Jonckheere–Terpstra test, $P = 0.03$) (Figure 4). No significant correlation existed between peak serum LA levels and mean change in sICAM-1 (Kendall's $\tau = -0.14$, $P = 0.27$).

Discussion

LA is a natural antioxidant that has been used to treat diabetic polyneuropathy and is an effective therapy for EAE, an animal model of MS.[21,22] This is the first report of a clinical trial of LA in MS. We demonstrated that oral LA was well tolerated and at a dose of 1200 mg resulted in measurable serum levels of LA in almost all patients. Importantly, despite the small sample size and relatively short duration of this pilot trial, our results suggest that LA is capable of decreasing two immunologic markers of MS activity, serum MMP-9 and sICAM-1, which are indirectly associated with T-cell migration into the CNS.

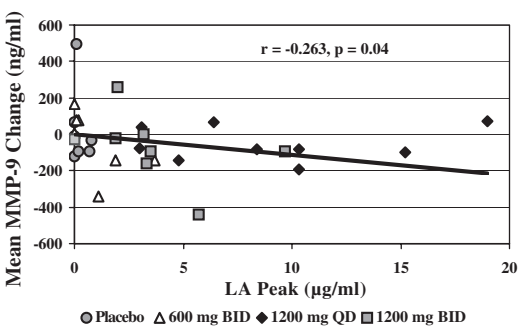

Figure 3 *Mean change in serum MMP-9 versus peak serum LA level. Each dot depicts one subject. The Y-axis represents the mean change in MMP-9 levels for the three visits (3, 4 and 5) as compared to the baseline value for each subject. The X-axis represents the peak serum values of LA in µg/mL. The slope of the line indicates a significant negative correlation between the peak serum LA values and the MMP-9 mean change (Kendall's $\tau = -0.263$, $P < 0.04$).*

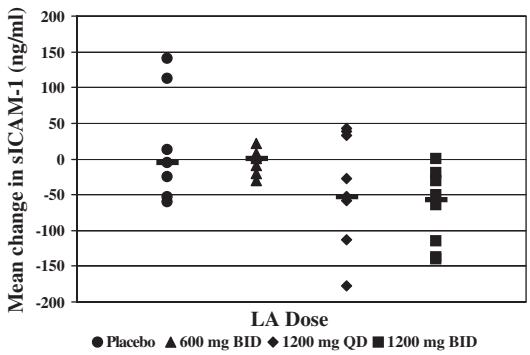

Figure 4 *Mean change in serum sICAM-1 versus LA dose. Each dot in the figure represents one subject. The Y-axis represents the mean change in sICAM-1 in ng/mL for visits 3, 4 and 5 as compared to the baseline value for each patient. The horizontal bar in each treatment group represents the median change in sICAM-1 levels for the group. A significant dose effect on changes in sICAM-1 levels was demonstrated using the Jonckheere–Terpstra test (P = 0.03).*

We chose to assess serum markers associated with T-cell migration and disease activity in MS based on our observations that LA inhibits the ability of T cells to travel into the CNS in EAE. Proteases, such as MMP-9 and adhesion molecules play a crucial role in MS pathogenesis by modulating T-cell migration into the CNS. Increased serum MMP-9 levels correlated with the appearance of new gadolinium-enhancing lesions in MS and treatment of MS patients with IFN-β1b decreased MMP-9 levels with a concomitant decline in new gadolinium-enhancing lesions.[31] Stüve *et al*. demonstrated that IFN-β1b antagonizes chemokine induction of MMP-9 at the level of transcription.[32] Our observations similarly revealed diminished serum MMP-9 following administration of LA as compared to baseline levels. How LA may decrease MMP-9 activity is uncertain, but there is evidence that LA can inhibit activation of proinflammatory transcription factors such as nuclear factor-kappa B (NF-κB).[33–35] NF-κB promotes the transcription of several genes coding for a number of proteins implicated in the pathogenesis of MS, including MMP-9 and ICAM-1.[36,37] In support of this, we recently demonstrated that LA inhibits MMP-9 mRNA production in human Jurkat T cells.[23]

Increased levels of soluble adhesion molecules in the serum such as sICAM-1 have been observed in patients with relapsing–remitting MS during the clinical relapse[39] and prior to the appearance of new gadolinium-enhancing lesions on MRI.[18,38] Increases in sICAM-1 may reflect increased endothelial cell activation in MS and other autoimmune diseases.[10–13] Paradoxically, some studies have shown that treatment of MS patients with IFN-β1b is associated with increases in sICAM-1.[46,47] How IFN-β1b increases serum sICAM-1 levels is unclear, but it is thought to proceed via proteolytic processing or by facilitating the generation of alternative splice-forms of ICAM-1 mRNA.[40] In contrast to IFN-β therapy, we found that increasing doses of LA resulted in reduced levels of serum sICAM-1. As LA appears to inhibit NF-κB activation,[33–35] we would expect LA to inhibit production

of ICAM-1 at the transcriptional level. We hypothesize that LA treatment inhibits expression of both endothelial cell membrane and soluble ICAM-1, thereby impeding T-cell migration into the CNS. However, we cannot exclude the possibility that LA might inhibit metalloproteinases responsible for proteolytic processing of membrane-bound forms of ICAM-1, thereby ameliorating the appearance of sICAM-1 in LA-treated patients.[41] Based upon our results, we propose that the combined effect of decreased MMP-9 activity and ICAM-1 expression could result in a blockade to trafficking of T cells into the CNS of MS patients, similar to what we have observed in EAE.[21–23]

While oral LA was generally well tolerated, there was considerable intersubject variability in absorption of LA as manifested by differences in peak serum levels and time to peak level. Variability in absorption of LA has been previously described.[42–44] Achieving an adequate serum level of LA is of importance as shown by the relationship between peak levels of LA and mean decrease in serum MMP-9. sICAM-1 changes did not correlate with peak serum levels of LA but correlated rather with total dose of LA. sICAM-1 levels decreased more with increasing doses of LA with 1200 mg BID causing more of an effect than 1200 mg QD. This suggests that changes in MMP-9 may relate to peak serum levels whereas changes in sICAM-1 may require more sustained blood levels of LA. This may imply different mechanisms of action. The significance of peak levels versus total dose of LA in affecting the putative biomarkers of MS needs further exploration. LA can be administered by intravenous infusion safely, as has been done in clinical trials for diabetic polyneuropathy, and represents one way of obtaining reliable serum levels.[26,45] Intravenous LA might prove to be an effective treatment for MS relapses. Alternatively, preparations of LA that are more reliably absorbed may result in more consistent blood levels and could potentially be used as an oral adjuvant therapy for MS.

In summary, oral LA represents a promising therapy for MS and warrants further investigation.

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