REVIEW

OPEN

Arginine therapy in mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes

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Purpose of review
We would like to inform clinicians that the systematic administration of oral and intravenous L-arginine is therapeutically beneficial and clinically useful for patients with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), when they maintain plasma arginine concentration at least 168 μmol/l.

Recent findings
MELAS is associated with endothelial dysfunction by decreased plasma L-arginine, nitric oxide (NO), and cyclic guanosine monophosphate. Endothelial dysfunction is also evident using flow-mediated vasodilation measurement by high-resolution Doppler echocardiography in the forearm artery in patients with MELAS. L-arginine is known to be an important precursor of NO to normalize the endothelial function in MELAS. In our clinical trial followed by 7 years follow-up study, the systematic administration of L-arginine to patients with MELAS significantly improved the survival curve of patients compared with natural history. Maintaining plasma arginine concentration at least 168 μmol/l may prevent the ictuses through the putative pathophysiologic mechanism and optimal normalization of endothelial dysfunction.

Summary
Neither death nor bedriddenness occurred during the 2-year clinical trials, and the latter did not develop during the 7-year follow-up despite the progressively neurodegenerative and eventually life-threatening nature of MELAS. Therapeutic regimen of L-arginine on MELAS may be beneficial and clinically useful for patient care with MELAS.

Keywords
endothelial dysfunction, L-arginine, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), stroke-like episodes, therapeutic regimen

INTRODUCTION

Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) is a progressively neurodegenerative and eventually life-threatening mitochondrial disorder that causes anatomohistopathological and clinical findings [1]. An A-to-G transition mutation at nucleotide position 3243 in mitochondrial DNA (m.3243A>G) is the most common cause of this syndrome [2]. MELAS is distinguishably characterized by the sudden, transient, and recurrent development of stroke-resembling symptoms (headache, nausea/vomiting, visual disturbance/visual field abnormalities, seizures, and impaired consciousness: ictus), with the distribution of brain lesions that are incongruent to the usual vascular territories [3]. The primary cause for stroke-like episodes (SLE) in patients with MELAS,

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Curr Opin Clin Nutr Metab Care 2020, 23:17–22

DOI:10.1097/MCO.0000000000000610

KEY POINTS

- Maintaining plasma arginine concentration at least 168 μmol/l may prevent the ictuses through the putative pathophysiologic mechanism and optimal normalization of endothelial dysfunction.
- The bedriddenness rate was 0% at the completion of both the 2-year clinical trials and the 7-year follow-up, in marked contrast to 5.2% in the 5-year cohort study.
- The mortality rates 0% in five patients with adult-onset, interictal MELAS who continued receiving oral L-arginine during the interictal phase and were appropriately treated with intravenous L-arginine during the acute phase and 50.0% in two patients with adult-onset, acute MELAS who were treated with intravenous L-arginine but were not treated with oral L-arginine during the interictal phase infer that this therapeutic regimen is appropriately rationalized to prevent the progression and fatal outcome of MELAS.
- Sudden death occurred in two patients with juvenile-onset MELAS, one each in the clinical trials of oral and intravenous L-arginine, implying the eventually life-threatening nature of MELAS.
- MELAS was well controlled in the 2-year clinical trials as indicated by a little change in the JMDRS scores, which is quite different from those in the clinical study for 7 years follow-up, indicated that disease progression might have been furthered by less rigorous clinical control.

whether mitochondrial cytopathy, angiopathy, or both, remains controversial.

We reported that MELAS is associated with endothelial dysfunction using endothelial-dependent vasodilatation methods by a high-resolution doppler echocardiography [4]. Because patients with MELAS have defective respiratory chain enzyme activities, a high NADH/NAD^+ ratio inhibits the nitric oxide (NO) synthetase reaction to cause a decreased production of NO at the endothelial cells or smooth muscle cells in the artery. In addition, asymmetrical dimethyl-arginine (ADMA), a risk factor of ischemic heart disorders, was relatively increased in patients with MELAS [5], which may lead to a negative effect on the endothelial NO synthetase activity. Indeed, decreased NO metabolite concentrations during SLE and lower NO synthesis rate were observed in patients with MELAS [5–8]. If hyperactive cytochrome *c* oxidase (COX) may decrease the regional NO concentration, all of the above scenarios lead to the segmental vasodilatation defect especially in the segment of strongly SDH-reactive blood vessel (SSV) regions in the cerebral artery or arterioles. These findings suggest that endothelial dysfunction because of NO deficiency is involved in the pathogenesis of SLE [9▪,10,11]. Low plasma concentration of L-arginine, a potent donor of NO, observed in patients with MELAS [5,8] supports the hypothesis and indicates the therapeutic potential of L-arginine on SLE.

Using L-arginine, we evaluated the potential at acute phase treatment by L-arginine infusion to cure the symptoms associated with SLE [4,5,12], and for prevent the SLE at intrinsic phase by L-arginine oral administration to decrease the severity of SLE, as the OL-MELAS Research Study[13▪].

STUDY DESIGN OF THE OL-MELAS RESEARCH

The OL-MELAS Research is a 9-year clinical study, which integrated the pooled data from two, 2-year, phase III, prospective, multicenter, open-label clinical trials of oral and intravenous L-arginine in patients with juvenile-onset or adult-onset MELAS; the research was conducted at 10 medical institutions in Japan, in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The protocols were approved by the institutional review board at each site. Oral L-arginine and intravenous L-arginine were purchased commercially. The clinical trials were registered at Center for Clinical Trials, Japan Medical Association (registry numbers: JMACTR-IIA00023 and JMACTR-IIA00025). We compared the results of 9-year clinical study with nationwide epidemiologic study on this patient group available in Japan, which revealed the natural course of patients with MELAS [13▪].

RESULTS OF THE CLINICAL TRIALS OF ORAL AND INTRAVENOUS L-ARGININE

In the clinical trial of oral L-arginine, before–after analysis revealed no statistically significant difference in the MELAS stroke scale, mitochondrial disease severity scores, or migraine severity scores. Namely, L-arginine therapy failed to achieve the endpoints. Nevertheless, the *P* value was 0.0549 in changes in the MELAS stroke scale, indicating a tendency of oral L-arginine to improve symptoms [13▪].

Regarding the efficacy of intravenous L-arginine, furthermore, the improvement rates of headache and nausea/vomiting at 2 h after completion of the initial intravenous administration were 25.0% (2/8) and 50.0% (3/6; $P = 1.0000$ each), respectively. Therefore, the statistical hypothesis that 'the rate of improvement is greater than 30%' could not be tested for these co-primary endpoints [13▪].

In the clinical trial of intravenous L-arginine study, the primary endpoints could not be achieved.

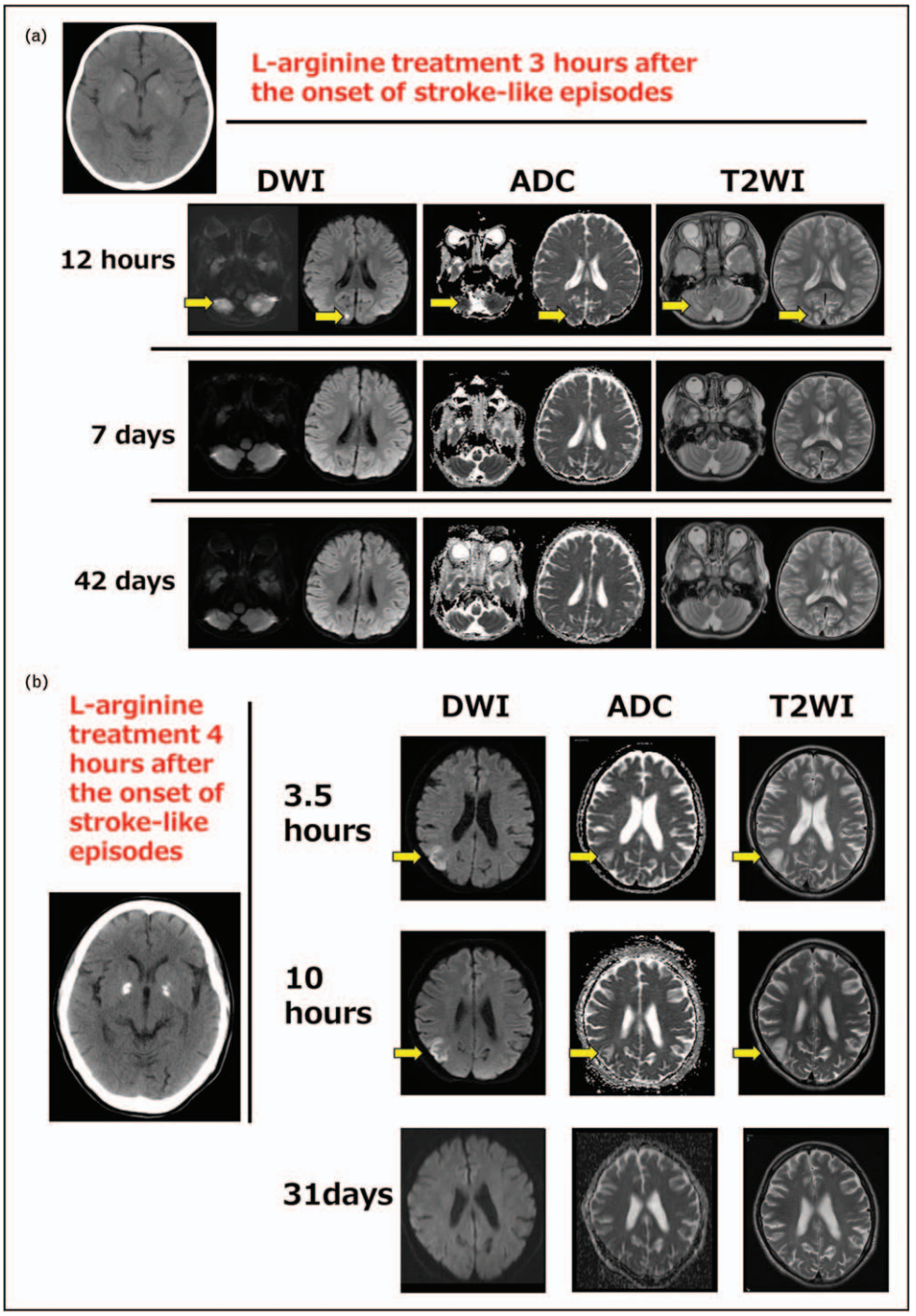

FIGURE 1. Serial brain MRI images in two patients with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) after treatment of L-arginine at superacute phase of (SLE). (a) A serial brain MRI was taken at 12 h, 7 days, and at 42 days after the onset of SLE in patient 1. Diffusion-weighted images (DWIs) showed high-intensity signal in bilateral cerebellar cortex and right posterior cortex (arrow), whereas mild-intensity signal in apparent diffusion coefficient (ADC), and low intensity in ADC. However those showing abnormal intensity in 12 h after onset of SLE showed normal intensity in DWI, ADC, and T2WI at 7 days and at 42 days after the episode. (b) A serial brain MRI was taken at 3.5 h, 10 h, and at 31 days after the onset of SLE in patient 2. T2WI showed mild high-intensity signal in cortex and subcortical white matter in the right parietal lobe (m), which becomes much less high-intensity signal in 3 days (p), and become completely normal in 1 month after the onset of SLE(s). DWI showed string-like gyriform-hyperintensity localized in the cerebral cortex, which harmonized with low-intensity signal on ADC map. These data suggested that cytotoxic edema exist in the cortex, on the other hand, vasogenic edema exist in the adjacent white matter. However, such MRI abnormalities are not recognized in MRI images at 1 month later from the onset of SLE without atrophic change. *Source:* They are the same as the original (reference [12]) and not adapted.

We speculate that the extended window for intravenous administration from '3 h' in our previous clinical study [5] to '6 h' in the present clinical trial might have disadvantageously attenuated the pharmacologic effects of intravenous L-arginine. Indeed, L-arginine infusion during the hyperacute phase of SLE in MELAS may significantly improve the symptoms, and also completely protect the progression of neuroimaging change from transient to permanent brain atrophy [12] (Fig. 1).

RESULTS OF THE 7-YEAR FOLLOW-UP STUDY

At the completion of the 2-year clinical trials, both the bedriddenness and mortality rates were 0% despite the progressively neurodegenerative and eventually life-threatening nature of MELAS. At the completion of 7-year follow-up, the bedriddenness rate remained to be 0%; in contrast, the mortality rates of patients with juvenile-onset or adult-onset MELAS were 22.2 and 0.0% in patients with interictal MELAS and were 12.5 and 50.0% in patients with acute MELAS. The 9-year survival of patients who were treated with oral and intravenous L-arginine is significantly improved with those seen in the natural course of MELAS who did not get treated with L-arginine [5] (Fig. 2). Consequently, the ictuses developed at a plasma arginine concentration of 167 μmol/l or below are recognized in our clinical trials [13▪].

OTHER CLINICAL STUDIES OF L-ARGININE THERAPY FOR STROKE-LIKE EPISODES

In addition to our OL-MELAS research, the eligibility criteria of which included patients carrying m.3243A>G, Ganetzky *et al.* [14▪] conducted a retrospective analysis of the therapeutic efficacy of intravenous L-arginine for SLE in nine pediatric patients harboring mitochondrial DNA mutations or nuclear DNA mutations except for m.3243A>G. Significant therapeutic benefit of L-arginine therapy was observed in these patients as well, which suggests that treatment with L-arginine is also favorable in patients with various genetic causes. Other clinical studies of L-arginine supplement therapy for MELAS have been done in different countries [15–21], and showed the improvement of the severity of the disease progression in MELAS.

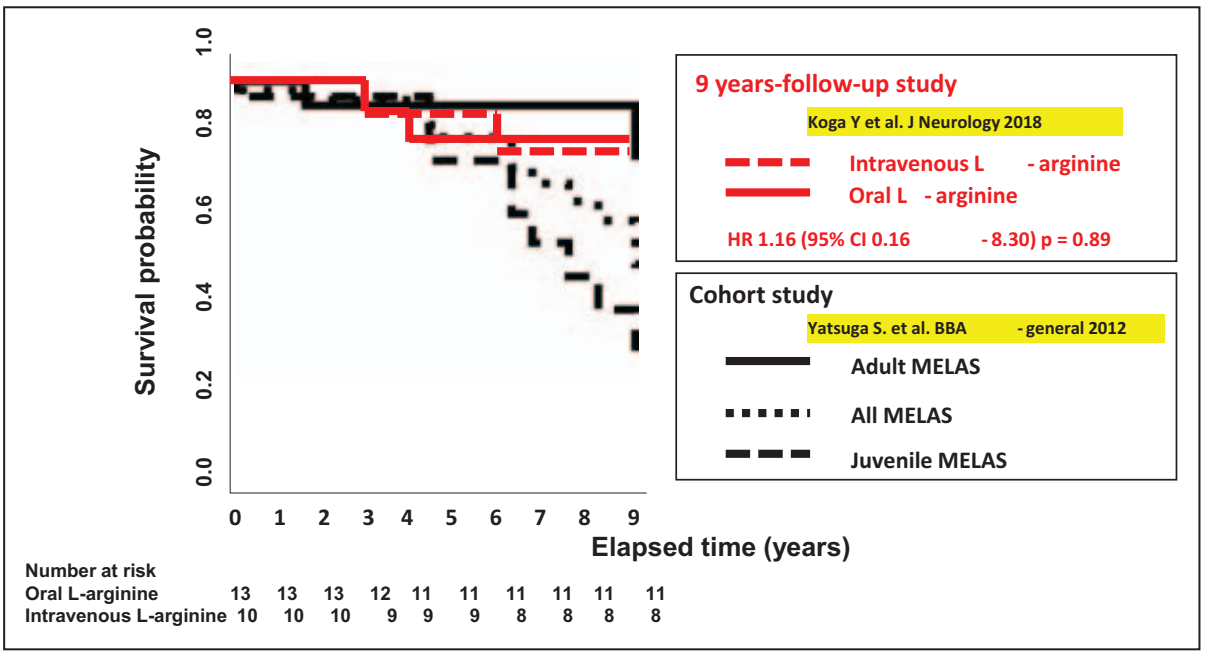

FIGURE 2. Comparison of survival curve in clinical trial with L-arginine and natural course without L-arginine in patients with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS). Black lines: A Kaplan–Meier survival curve is shown in the cohort study and adopted (Reference [13▪]). The dashed line indicates the juvenile form and the solid line indicates the adult form. The results of the log-rank analysis were significant. The juvenile form was associated with a higher risk of mortality than the adult form (hazard ratio, 3.29; 95% CI, 1.32–8.20). Those survival curves are recalculated with normalized severity score of the patients in the clinical trial study. Red lines: A Kaplan–Meier survival curve is shown in the clinical trial and 7 years- follow up study (Reference [13▪]). The dashed line indicates the intravenous study and the solid line indicates the oral study. The surviving curve in clinical trial is significantly improved with those seen in the natural history. *Source:* They are the same as recalculated from the original (Reference [13▪]).

Additionally, several case studies showing the therapeutic efficacy of L-arginine on SLE have been reported. Especially, Hovsepian *et al.* [22] performed magnetic resonance spectroscopy (MRS) to access the metabolic change in a SLE lesion of patients with MELAS before and after intravenous L-arginine therapy. Serial MRS showed decreased lactate peak and increased *N*-acetyl aspartate concentration (i.e. neuronal density) after treatment, which corresponded with clinical improvement.

PHARMACOLOGICAL EFFECTS OF L-ARGININE ON THE PATHOPHYSIOLOGICAL MECHANISM OF STROKE-LIKE EPISODES DEMONSTRATED BY USING MOLECULAR IMAGING

The recent progress of molecular imaging with positron emission tomography (PET) and magnetic resonance imaging (MRI) has shed light on the pathophysiological process of SLE and the pharmacological effects of L-arginine.

Brain MRI and single-photon emission computed tomography (SPECT) imaging usually exhibit cerebral hyperemia, vasodilatation, and vasogenic edema because of mitochondrial angiopathy in acute lesions of SLE. In addition, arterial spin labeling (ASL) perfusion MRI clearly shows serial changes in cerebral perfusion related to the disease activity of SLE. Interestingly, we discovered regional cerebral hyper perfusion on ASL imaging in the preclinical phase of SLE in some patients with MELAS [23] (Fig. 3). These hyperperfused areas developed into acute lesions at the clinical onset of SLE, suggesting that subclinical SLE may have already occurred several months prior to the clinical onset. Because L-arginine can accommodate endothelial dysfunction (i.e. mitochondrial angiopathy), L-arginine therapy is expected to prevent the progression to evident SLE.

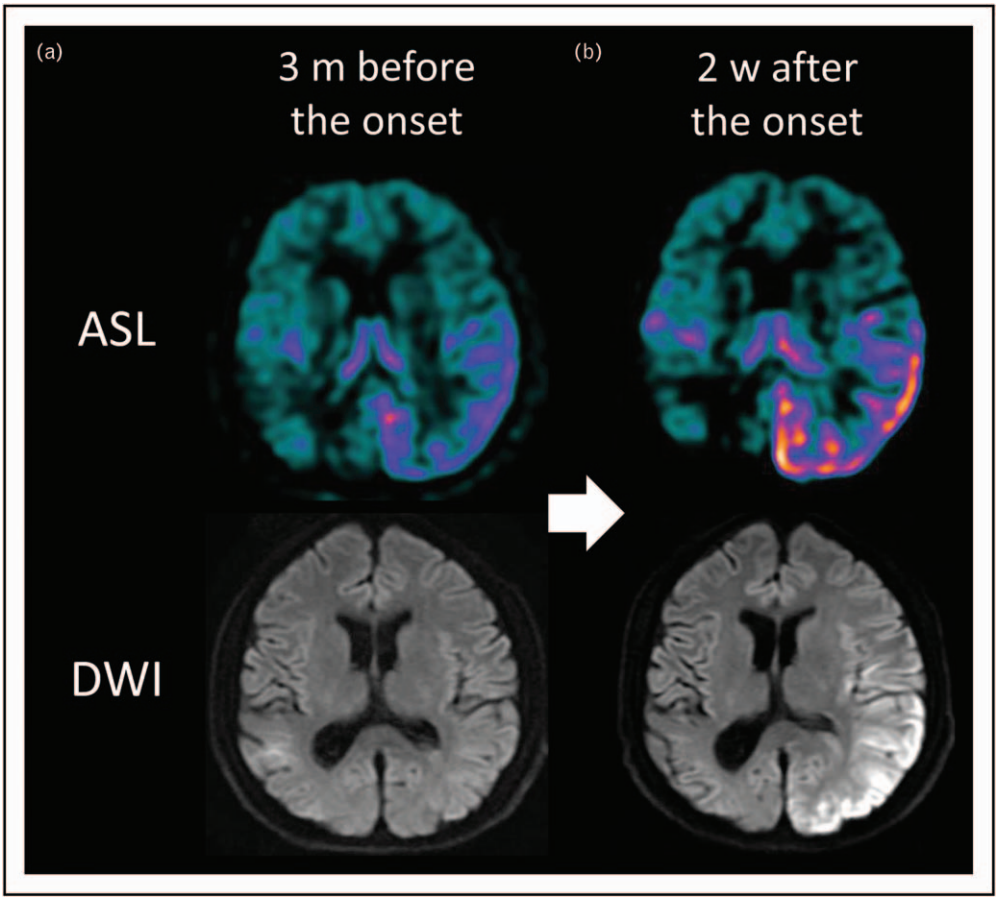

FIGURE 3. Representative serial brain MRI images of a patient with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) that shows preclinically latent hyperperfusion because of SLE. (a) MRI images 3 months before the clinical onset of the SLE (preclinical phase) demonstrate regional hyperperfusion in the arterial spin labeling (ASL) images in the left temporo-occipital lobe. Meanwhile, the diffusion-weighted images (DWIs) showed apparent normalcy in this area. (b) MRI images 2 weeks after the clinical onset of the SLE (acute phase) show an acute lesion in the DWIs in the left temporal lobe with hyperperfusion in the ASL images. *Source:* They are adapted from the original (reference [23]).

In addition to a therapeutic effect on mitochondrial angiopathy as a precursor of NO, L-arginine has a facilitative effect on tricarboxylic acid (TCA) cycle metabolism (i.e. mitochondrial cytopathy) via a conversion to α-ketoglutarate. Our previous study using ^{11}C-acetate PET showed that intravenous administration of L-arginine enhanced TCA cycle kinetics in patients with mitochondrial cardiomyopathy carrying m.3243A>G [24]. The dual pharmacological effects of L-arginine on both mitochondrial angiopathy and cytopathy may have improved the long-term prognosis of patients with MELAS.

CONCLUSION

The OL-MELAS Research is therapeutically unique in the following achievements: a) specification of the plasma arginine concentration (trough level: 168 μmol/L) at which L-arginine can prevent an ictus that may cause irreversible brain damage, or bedriddeness in the worst case; b) presentation of '0%' in the bedriddenness rate during the strict systematic administration of oral and intravenous L-arginine in the 2-year clinical trials; and c) the obvious suppression and modest retardation of disease progression at the ends of 2-year clinical trials and 7-year follow-up, respectively [13▪]. The L-arginine therapy is listed in the recent review of patient care standards for primary mitochondrial disease [25▪].

Acknowledgements

We would like to thank Dr Eisuke Inoue for his statistical analysis in the study.

Financial support and sponsorship

This study was funded by the Ministry of Health, Labour and Welfare of Japan (H14-Shouni-006) and Center for Clinical Trials, the Japan Medical Association Center (CCT-B-1803, CCT-C-2001).

Conflicts of interest

There are no conflicts of interest.

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- ▪▪ of outstanding interest

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