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CLINICAL TRIAL

High doses of biotin in chronic progressive multiple sclerosis: A pilot study

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KEYWORDS
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Abstract
Background: No drug has been found to have any impact on progressive multiple sclerosis (MS). Biotin is a vitamin acting as a coenzyme for carboxylases involved in key steps of energy metabolism and fatty acids synthesis. Among others, biotin activates acetylCoA carboxylase, a potentially rate-limiting enzyme in myelin synthesis.
Objectives: The aim of this pilot study is to assess the clinical efficacy and safety of high doses of biotin in patients suffering from progressive MS.
Study design: Uncontrolled, non-blinded proof of concept study
Methods: 23 consecutive patients with primary and secondary progressive MS originated from three different French MS reference centers were treated with high doses of biotin (100-300 mg/day) from 2 to 36 months (mean=9.2 months). Judgement criteria varied according to clinical presentations and included quantitative and qualitative measures.
Results: In four patients with prominent visual impairment related to optic nerve injury, visual acuity improved significantly. Visual evoked potentials in two patients exhibited progressive

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reappearance of P100 waves, with normalization of latencies in one case. Proton magnetic resonance spectroscopy (H-MRS) in one case showed a progressive normalization of the Choline/Creatine ratio. One patient with left homonymous hemianopia kept on improving from 2 to 16 months following treatment's onset. Sixteen patients out of 18 (89%) with prominent spinal cord involvement were considered as improved as confirmed by blinded review of videotaped clinical examination in 9 cases. In all cases improvement was delayed from 2 to 8 months following treatment's onset.

Conclusions: These preliminary data suggest that high doses of biotin might have an impact on disability and progression in progressive MS. Two double-blind placebo-controlled trials are on going.

1. Introduction

In 85% of cases, multiple sclerosis (MS) initially evolves through relapses that resolve completely or incompletely (relapsing-remitting MS or RRMS). However, in about 50% of cases, patients who were initially presented with a relapsing-remitting course subsequently evolve towards a progressive form (secondary progressive MS or SPMS). In 15% of patients, the disease strikes with an immediate progressive setting without relapses (primary progressive MS or PPMS). It is possible to distinguish two components in the pathophysiology of multiple sclerosis: (1) an inflammatory component, responsible for the attacks, and (2) a degenerative component characterized by progression with few or less inflammation (Noseworthy et al., 2000; Compston and Coles, 2008). Among hypotheses that tend to explain the cause(s) of progressive MS, it has been proposed that progressive degeneration could be linked to a phenomenon of "virtual hypoxia" caused by a mismatch between increased energy demand by the demyelinated axon and decreased energy production because of mitochondria injury (Luessi et al., 2012; Stys et al., 2012; Witte et al., 2013).

While immunosuppressive or immunomodulatory therapies reducing the inflammatory reaction are mainly effective at the relapsing-remitting phase of the disease, as they decrease the number or the duration of relapses and lesion accumulation on MRI, they only have poor efficacy on the long-term disability and only weak or no effectiveness in the progressive (primary or secondary) forms of the disease (Hauser et al., 2013). With regards to permanent disability, fampridine is the only approved symptomatic drug that improves walking speed in a subgroup of patients (Goodman et al., 2009). Up to now, no drug has been found to have any impact on the disease's progressive phase (primary or secondary).

Biotin (or vitamin H) is a ubiquitous water-soluble vitamin that is naturally found in many foods (Zempleni and Mock, 1999). In mammals, biotin acts as a coenzyme for carboxylases involved in key steps of energy metabolism and fatty acids synthesis.

High doses of biotin have been found to be a therapeutic option in "biotin responsive basal ganglia disease" (BBGD; OMIM 607483), an orphan neuro-metabolic disease caused by mutations in the SLC19A3 gene coding for a thiamine transporter (Tabarki et al., 2013). Patients with BBGD display severe episodes of Leigh-like encephalopathy leading to death or permanent disability and show dramatic improvement when high doses of biotin (5–10 mg/kg/day) and thiamine are administered (Tabarki et al., 2013). In addition, we recently found that 5 patients suffering from optic neuropathies and leukoencephalopathy did respond clinically to high doses of biotin (Sedel et al., 2011). Subsequently, we discovered that one of these 5 patients suffered from SPMS. From this starting point, high doses of biotin were tested in 22 additional patients with PPMS and SPMS. Results are reported here.

2. Methods

Twenty-three consecutive patients with PPMS or SPMS were treated with high doses of biotin ranging from 100 mg to 600 mg/day (median = 300 mg/day divided in three doses) in a compassionate use open-label trial. All patients were followed in French MS reference centers and fulfilled the McDonald criteria for Multiple Sclerosis (Polman et al., 2011) and Lublin revised criteria for progressive MS (Lublin et al., 2014). The term progressive disease refers to steadily increasing objectively documented neurologic dysfunction/disability without unequivocal recovery (fluctuations and phases of stability may occur, Lublin et al., 2014). All patients included in this study had a disease progressing for at least the last 12 months. None of the patients displayed any acute exacerbation within the 6 months prior to treatment's onset. The study has received review from an ethics committee. Seventeen patients were followed at Pitié-Salpêtrière Hospital by FS and CP; 3 patients were followed in Reims Academic Hospital by AT and 3 patients were followed in Nice Academic Hospital by CLF. Most of them had been treated with drugs usually proposed in progressive MS that failed to improve their neurological condition including monthly pulses of IV methylprednisolone (IVMP), cyclophosphamide, azathioprine or fampridine (Table 1 and Supplementary data). None of these drugs were introduced during the period of treatment with biotin except consecutive pulses of IVMP in case of MS relapses.

Assessment of biotin's efficacy varied according to the clinical situation and the center. In the four patients with chronic optic neuropathies, efficacy was assessed using visual acuity (VA), Goldmann perimetry and/or visual evoked potentials (VEP). In the patient with homonymous hemianopia, efficacy was assessed using Humphrey automated perimetry. In the 18 patients with spinal cord involvement, efficacy was assessed using walking distance, EDSS, TW25,

muscle strength testing and videotaped clinical examination in a subset of patients. In addition, clinicians collected the following clinical symptoms: fatigue, swallowing difficulties, dysarthria, Uhthoff's phenomenon and urinary dysfunction.

For 9 patients, videotaped clinical examinations were reviewed blindly by an independent examiner from a center not participating in the study and specialized in the evaluation of motor disability in multiple sclerosis and related neurodegenerative diseases (Dr. Kathleen Zackowski, Kennedy Krieger Institute/Johns Hopkins School of Medicine, Baltimore, USA). Before and after treatment videos were mixed up and numbered 1 or 2. No visual or auditory details in the video could help identify if the patient was filmed before or after treatment initiation. The blinded examiner was asked to rate the muscular movements and walking exercises, to select a video where the patient was eventually improved and the degree of improvement, based on clinical global impression. Two videos were reviewed per patients corresponding to (1) the first available video before or soon after treatment's onset when the pre-treatment video was lacking and (2) the latest available video after treatment's onset. The examiner who performed the rating was aware of the uncontrolled open label nature of the study. Capsules containing 100 mg of biotin each were prepared by the pharmacy of Pitié-Salpêtrière Hospital *(Patent Application WO/2011/124571)*.

3. Results

Twenty-three patients aged from 26 to 75 years (mean=52.8 years) were treated with high doses of biotin from 2 to 36 months (mean treatment's duration=9.2 months). Fourteen patients suffered from PPMS and 9 from SPMS. Four patients (1–4) had permanent visual loss following optic nerve involvement; one patient (patient 5) had progressive lateral hemianopia caused by involvement of optic radiations and 18 patients (patients 6–23) had progressive paraparesis or tetraparesis related to spinal cord involvement. Results are summarized in Table 1, Figs. 1–3 and are detailed in Supplementary data.

3.1. Patients with prominent optic nerve involvement (1–4)

The 4 patients with chronic visual loss related to involvement of optic nerves exhibited improvement of VA after a delay of 3 months following treatment's onset. The 8 diseased eyes improved by a mean of 0.18 decimal units (sd=0.11) a threshold considered beyond a possible test-retest effect (Rosser et al., 2003). In all cases, improvement was observed with the dose of 300 mg/day. Improvement was maintained over time except in patient 2 who exhibited worsening after a MS relapse (supplementary data). Improvement of VA was associated with improvement of Goldmann perimetry in patients 2–4 (not shown). In addition, VEP were performed in patients 1 and 4. In both cases, P100 waves, although not recordable or very delayed at baseline were clearly observable after 9 months of treatment with normalization of latencies in patient 2 (Fig. 1).

H-MRS was performed every 3 months from treatment's onset in patient 1 (Fig. 2). A progressive normalization of the Choline/Creatine ratio, a myelin marker, was observed over time. After 9 months of treatment, this ratio had returned to normal values.

3.2. Patient with homonymous hemianopia (patient 5)

Patient 5 had progressive left homonymous hemianopia worsening over 4 years, related to a lesion involving optic radiations (Fig. 3). Humphrey perimetry was observed to continuously improve from M2 (2 months after treatment's initiation) up to M16 (last follow-up). This was confirmed by Visual field defect's quantification showing better mean deviation (MD) values reached after 7 months of treatment compared to those obtained during the 4 years of the pre-treatment follow-up (Fig. 3). Despite functional improvement, no change in the size or aspect of the right occipital white matter lesion was noted on brain MRIs performed after treatment's onset.

3.3. Patients with spinal cord involvement (6-23)

Eighteen patients had prominent involvement of the spinal cord with progressive tetraparesis (11 cases) or paraparesis (7 cases). Sixteen out of 18 patients (89%) displayed clinical improvement after a delay ranging from 2 to 8 months. Seven patients (6, 7, 8, 13, 14, 15, 16) started to improve with 100 mg/day of biotin; 5 of them (6, 8, 14, 15, 16) improved even more after increasing the dosage to 300 mg/day. In the 9 remaining patients, improvement started at the dose of 200 mg (patients 21 and 22) or 300 mg/day (patients 9, 11, 17, 18, 19, 20, 23). In 9 cases (50%), improvement was documented by blinded review of videotaped clinical examinations (Table 2). In all cases, the blinded examiner recognized that the latest video (after the longest period of treatment) corresponded to the best examination.

In the sub-group of 11 patients with tetraparesis, 9 patients improved after a delay of 2–8 months. The longest period of observation in this group was 12 months (patient 6). Two patients (10 and 12) did not respond at all to treatment despite increasing the dosage to 300 mg/day. In patient 21, treatment's withdrawal for 15 days was followed by a marked worsening leading to the reintroduction of the treatment.

In the sub-group of 7 patients with paraparesis, all patients improved after a delay ranging from 2 to 5 months. The TW25 test was performed in 5/7 cases and best improvement compared to baseline ranged from 17.6% to 33.8%, which can be considered as clinically relevant (Goodman et al., 2009; Schwid et al., 2002). In patients 7 and 20, although some improvement was observed after 3 months of treatment, the positive effect was not maintained overtime. Worsening occurred following a MS relapse in patient 7 and without any explanation in patient 20.

3.4. Other symptoms improvement

Neurologists considered that the following symptoms and signs also improved: fatigue (5 cases), swallowing difficulties (4 cases), dysarthria (3 cases), sensory signs (2 cases), gait ataxia (2 cases), urinary dysfunction (2 cases),

Table 1 Efficacy data in 23 patients with progressive MS treated with high doses of biotin.

Case number (location)	Age (yrs)	MS form	EDSS at onset	Prog disease duration (yrs)	Clinical form	Max dose (mg)	Total (mo)	Delay (mo)	MS Relapse	Quant improv	Qual improv	Efficacy: sustained (+), transient (+/−), no (−)	Additional MS therapies at baseline	Past MS treatments
1 (PSL)	74	SP	4	3	ON	600	34	3	−	VA (R: 0.1 (20/200)→0.3 (20/60); L: 0.2 (20/100)→0.5 (20/40)); VEPs, H-MRS	Psychiatric signs	+	None	MP pulses
2 (PSL)	26	SP	4	3	ON	300	18	3	+	VA (R: 0.1 (20/100)→ 0.3 (20/60); L: 0.3 (20/60)→0.6 (20/30)), perimetry		+/−	IFN	MP pulses
3 (PSL)	33	PP	4	9	ON	300	15	3	+	VA (R: 0.07 (20/250)→ 0.12 (20/160); L: 0.06 (20/320)→0.1 (20/200)),perimetry	Cognition, ataxia	+	None	MP pulses IFN
4 (PSL)	44	SP	4	4	ON	300	12	3	−	VA (R: 0.3 (20/60)→ 0.4 (20/50); L: 0.025 (20/400)→0.3 (20/60)), VEPs, perimetry	Uhthoff, fatigue, ataxia	+	None	MP pulses IFN Fingo
5 (PSL)	29	SP	3	4	LHH	300	16	2	−	Perimetry	Sensory symptoms	+	None	MP pulses
6 (PSL)	73	SP	8.5	12	Tetra-paresis	300	12	2	−	Muscle strength, EDSS: 8.5→8	Video, swallowing, dysarthria, sensory signs	+	None	MP pulses Cyclo
7 (PSL)	62	PP	7	8	Para-paresis	300	6	3	+	EDSS: 7→6.5, TW25: −31.8%	Video	+/−	None	MP pulses MPM
8 (PSL)	53	PP	5.5	3	Para-paresis	300	7	3	−	EDSS: 5.5→4, TW25: −32.6%	Video, dysuria	+	None	MP pulses
9 (PSL)	73	PP	8.5	20	Tetra-paresis	300	6	2	−	Muscle strength	Video, dysarthria, swallowing	+	None	NA
10 (PSL)	47	PP	8.5	21	Tetra-paresis	300	8	Not improved	+	Not improved	Not improved	−	None	

														MP pulses Cyclo Metho
11 (PSL)	49	PP	8.5	11	Tetra-paresis	300	8	8	−	Muscle strength	Video, dysarthria, swallowing	+	None	MP pulses IFNAza Cyclo
12 (PSL)	48	PP	8.5	10	Tetra-paresis	300	7	Not improved	−	Not improved	Not improved	−	None	Cyclo
13 (Reims)	41	SP	8.5	15	Tetra-paresis	300	6	3	−	None	swallowing, oscillopia, coordination	+	None	IFN MP pulses Cyclo
14 (Nice)	60	SP	6.5	6	Para-paresis	300	9	3	−	EDSS: 6.5→6	walking distance, fatigue	+	None	MP pulses
15 (Nice)	50	PP	8.5	20	Tetra-paresis	300	9	3	−	Muscle strength	trunk hypotonia	+	NTZ	Aza MPM Cyclo Mito
16 (Nice)	53	SP	9	13	Tetra-paresis	300	9	3	−	Muscle strength	trunk hypotonia	+	IgIV	IgIV Cyclo MPM Aza
17 (PSL)	75	SP	6	5	Para-paresis	300	6	4	−	TW25: −42% EDSS: 6→5.5	Video	+	None	MP pulses Stem cells
18 (PSL)	46	PP	6.5	30	Para-paresis	300	2	2	−	None	Fatigue, walking	+	None	MP pulses Aza
19 (PSL)	59	PP	6	10	Para-paresis	300	6	4	−	TW25: −33.8%, Muscle strength	Video	+	None	Cyclo
20 (PSL)	52	PP	3.5	5	Para-paresis	300	6	3	−	TW25: −17.6%,	Video, urgent mictions	+/−	None	NA
21 (Reims)	49	PP	8.5	6	Tetra-paresis	200	2	2	−	None	Fatigue	+	Fampridine	MP pulses Cyclo
22 (PSL)	63	PP	8.5	10	Tetra-paresis	200	5	2	−	Muscle strength	None	+	NTZ	NA
23 (Reims)	57	PP	8.5	13	Tetra-paresis	300	4	4	−	Muscle strength	Video, fatigue	+	None	MP pulses
Total	Mean=52.8	PP=60%		Mean=10.5		Med=300	Mean=9.2	Mean=3.1	4 /23 (17.4%)			(+): 78.3% (+/−): 13% (−): 8.7%		

Age: age at last follow-up; AZA: azathioprine; Cyclo: cyclophosphamide; Delay: treatment duration before first signs of improvement; Fingo: fingolimod; HH: homonymous hemianopia; H-MRS: proton magnetic resonance spectroscopy; IFN: β-interferon; IgIV: intravenous immunoglobulins, Max dose: maximum dose received per day; Med: median; Metho: methotrexate; MP: methylprednisolone; MPM: mycophenolate mofetil; Mito: mitoxantrone; Mo: months; MS relapse: any relapse of multiple sclerosis during treatment, NA: not available; NR: not relevant; NT: not tried, NTZ: natalizumab; ON: optic neuropathy; PP: primary progressive, Prog disease duration: duration of the progressive disease before treatment's onset; PSL: Pitié-Salpêtrière Hospital; Quant improve: quantitative measures improved; Qual improve: qualitative improvement; SP: secondary progressive, Spinal: spinal cord involvement; Sustained: sustained efficacy at last follow-up; Total: total treatment duration at last follow-up; VA: visual acuity expressed in decimals units and US Snellen equivalent before and after treatment (best recorded values) for right (R) and left (L) eye, VEPs: visual evoked potentials, Video: filmed clinical examination follow-up.

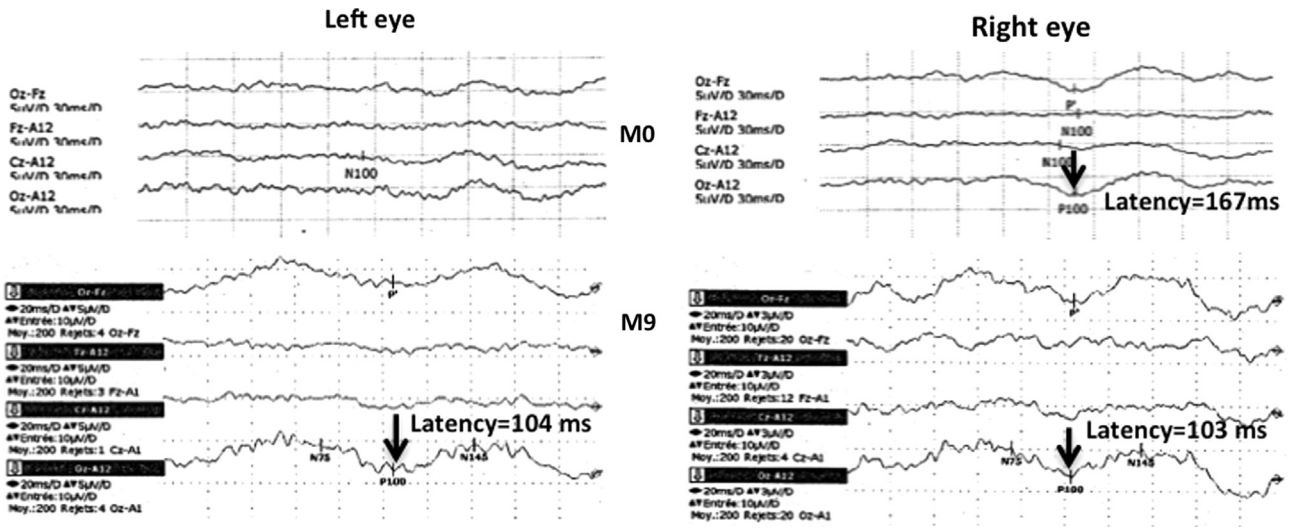

Fig. 1 Evolution of visual evoked potentials in patient 4 (whole field, 24 × 32, mean curves). On the left side, although no individualized P100 wave could be observed on the left before treatment (M0), a P100 wave was well confirmed after 9 months (M9) with a normal latency of 104 ms. On the right side, a P100 was obtained before treatment with an increased latency (167 ms). At M9, the latency of the P100 was in the normal range (103 ms).

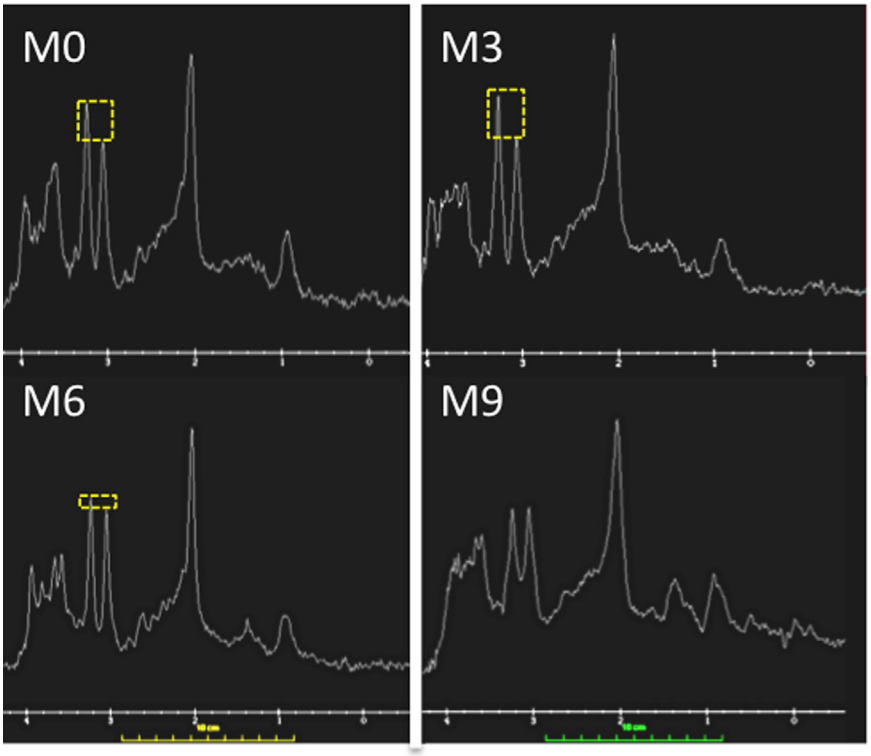

Fig. 2 Evolution of NMR spectroscopy at short echo time in the diseased brain white matter of patient 1. Note the progressive decrease in the choline/creatine ratio (materialized by a dotted square) starting after 6 months of treatment (M6) and normalization after 9 months (M9). Serial brain H-MRS were performed on a 3 T MR unit (General Electric, WI) with a single voxel acquisition using the PRESS sequence at short (TR=1500 ms, TE=28 ms) and long (TR=1500 ms, TE=135 ms) echo times in the white matter of the centrum ovale. The volume of interest was similarly located in all acquisitions and measured 40 mm × 16 mm × 20 mm (*x*,*y*,*z* axes).

cognition (1 case), psychiatric signs (1 case), oscillopia (1 case) motor coordination (1 case) and Uhthoff's phenomenon (1 case). Overall, the EDSS score significantly improved in 4/23 patients (22%) with a decrease of at least 1 point (for initial EDSS between 4 and 5.5) or of at least 0.5 (for initial EDSS between 6 and 8.5).

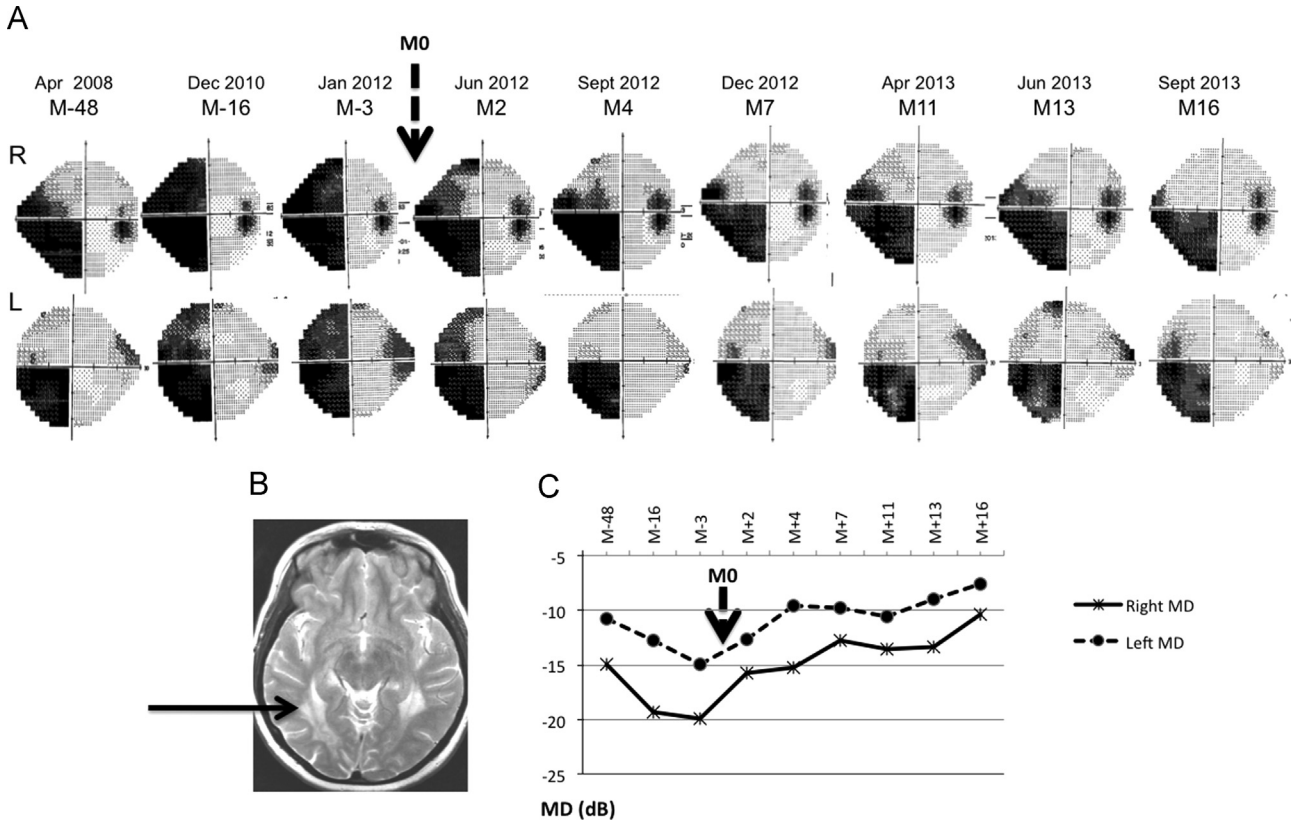

Fig. 3 Patient 5. (A) Follow-up of visual fields measured by Humphrey automated perimetry before treatment (between Apr 2008 and Jan 2012) and after treatment with 300 mg/day of Biotin (June 2012 to september 2013). Treatment was started on Apr 2012. Note the progressive left homonymous hemianopia (Black color) between Apr 2008 and Jan 2012 that improves (becoming progressively brighter) after two months of treatment (Jun 2012). Improvement was even more pronounced at M4, M7, M11, M13 and M16. (B) Location of the lesion involving the right optic radiations on brain MRI using a T2-weighted sequence. (C) Evolution of mean deviations of the visual field defects (MD) during the 48 months preceding treatment's onset and during the 16 months following treatment's onset. The progressive improvement of the MD values confirms the qualitative improvement shown in Fig. 3A. R: right eye; L: left eye.

3.5. Effect on relapses

Four patients (2, 3, 7 and 10) out of 23 (13%) receiving high doses of biotin experienced at least one MS relapse. This frequency was similar to that observed before treatment in these patients.

3.6. Safety data

No adverse effects were reported in 20 cases. Transient diarrhea was noted in 2 patients. Patient 1 died from cardiac failure 36 months after treatment's onset. Mild aortic valvulopathy with dilatation of the ascending aorta together with a first-degree atrio-ventricular block was discovered during the patient's follow-up. No relation was established between treatment's onset, mild cardiac abnormalities and death: no change in ECG or cardiac ultrasonography could be noted in this patient (Supplementary data). Patient 15 died one year after treatment's onset from a pneumopathy few days after sigmoid volvulus surgery. No relation could be established between death and treatment.

4. Discussion

Our results based on clinical, electrophysiological and H-MRS data suggest that high doses of biotin have some impact on disease progression and permanent disability in patients with progressive MS. Overall, 21/23 patients (91.3%) exhibited some qualitative or quantitative clinical improvement with high doses of biotin. Similar positive results were noted in patients with SPMS and PPMS suffering from optic neuropathies, homonymous hemianopia or spinal cord involvement. In all cases, clinical improvement was delayed by 2–8 months (mean=3 months) following treatment's onset. Only 2 patients with severe tetraparesis did not show any response to treatment, probably related to its short duration (8 and 7 months respectively). Indeed, in patient 11 with a severe tetraparesis, treatment's benefit only started 8 months after treatment's initiation. The range of dose was determined empirically after having observed some clinical improvement in a single patient with 300 mg/day of biotin. Several attempts to decrease or increase the dosage were performed. Increasing the dose to 600 mg/day in one patient was not associated with additional benefit whereas decreasing the dose to 100 mg/day in one patient was associated

Table 2 Results of blinded evaluation of videos.

Patient number	Initial video	Last video	Test	Score at initial visit	Score at last visit	Best examination in the opinion of examiner	CGI at best examination	Comments from the examiner
6	M4	M12	Deltoid (L)	4	4	M12	3	In video number 2 movements in right brachialis and extensor digitorum were more apparent
			Flexor digitorum profundis (L)	1	1			
			Brachialis (R)	0	2+			
			Extensor digitorum (R)	0	2+			
			Quadriceps femori (R)	1	2−			
			Quadriceps femori (L)	1	2−			
7	M0	M3	Time to make the same distance	101 s	41 s	M3	3	
8	M0	M7	Time to make the same distance	14 s	9 s	M7	3	Walk was faster in video 2
9	M0	M6	Flexor digitorum profundis (R)	2−	2	M6	3	
			Extensor digitorum (R)	2	2			
			Brachialis (R)	2−	2−			
			Triceps (R)	1	2−			
11	M3	M8	Flexor digitorum profundis (L)	0	2−	M8	3	
			Extensor digitorum (L)	0	2−			
			Brachialis (L)	2−	2−			
			Triceps (L)	2−				
			Extensor carpi (L)	0	2−			
			Flexor carpi (L)	0	2−			
17	M0	M7	Time to make the same distance	28	15	M7	3	The walking path in video 2 (M0) is more cluttered than the path in video 1 (M7), this will affect the speed of walking in the trial
19	M2	M6	Time to make the same distance	20 s	13 s	M6	2	
			Deltoid (R)	2−	2			

20	M0	M3	Time to make the same distance	44 s	35 s	M3	3	
23	M0	M3	Deltoid (L)	2−	2+	M3	3	

CGI (clinical global impression): Rate total improvement whether or not, in your judgement, it is due entirely to drug treatment. Compared to the worst video, how much has he changed? 0 = Not assessed; 1 = Very much improved; 2 = Much improved; 3 = Minimally improved; 4 = No change; 5 = Minimally worse; 6 = Much worse; 7 = Very much worse.

with worsening. In 5 cases, increasing the dosage from 100 to 300 mg/day was followed by an additional improvement. From these observations, the dose of 300 mg/day was thought to be associated with the best clinical response. In addition, the treatment appeared to be safe: transient diarrhea, the only minor adverse effect, was noted in 2 patients. Two patients died in the course of the trial but in both cases, death could not be attributed to the treatment.

Although these data rely on an open label study with the possibility of a placebo effect, they are in marked contrast with the natural history of progressive forms of MS where almost no spontaneous or sustained improvement occurs (Confavreux et al., 2000). Furthermore, clinical treatment's efficacy was confirmed in few patients by unbiased quantitative measures such as VEP and H-MRS that both showed continuous improvement during the first year of treatment. The normalization of VEP latencies and of the choline/creatine ratio suggests the possibility of myelin repair that would also account for the delayed efficacy. In contrast, the treatment is unlikely to be associated with an anti-inflammatory effect. Indeed, biotin did not prevent from inflammatory attacks as observed in 4 patients who displayed relapses while on treatment. On the other hand, the question whether high doses of biotin could favor attacks of the disease still remains. Of note, in our study, the rate of relapses did not significantly change before and after treatment but more data are still needed.

To our knowledge, high doses of biotin have never been hypothesized as a potential treatment for MS. The discovery that this might represent a therapeutic option in chronic progressive MS both in its secondary and primary forms relies on a serendipity. However *a posteriori,* the observed effects do rely on a strong rational. Progression in MS (either secondary or primary) is often considered as a consequence of both demyelination and energy failure (Luessi et al., 2012; Stys et al., 2012). A large proportion of ATP produced in the nervous system is used by the NA/K ATPase to restore the membrane resting potential. In the normal condition, myelin insulation reduces the energy demand during impulse propagation because only the nodes of Ranvier are excited. In contrast, in unmyelinated fibers where the entire membrane is involved, much more ATP is needed for ion pumping. As a consequence, it has been estimated that an unmyelinated axon may use up to 5000 times more energy than a myelinated axon (Quarles et al., 2006). In MS, in addition to the fact that demyelinated fibers increase their energy demand, energy production may be compromised because of mitochondrial injury (Witte et al., 2013). The resulting mismatch between increased energy demand for nerve conduction and decreased supply by impaired mitochondria could bias demyelinated axons towards a state of 'virtual hypoxia' culminating in degeneration (Luessi et al., 2012; Stys et al., 2012).

Biotin is a water-soluble vitamin that serves as an essential coenzyme for carboxylases catalyzing the transfer of a carboxyl (COOH) group to targeted substrates (Zempleni and Mock, 1999). The five biotin-dependent carboxylases are: pyruvate carboxylase (PC), propionly-CoA carboxylase (PCC), β-methylcrotonyl-CoA caboxylase (MCC), and acetyl-CoA carboxylase (ACC), with the latter enzyme existing in two distinct isoforms one of which is in the cytosol (ACC1) and the other is attached

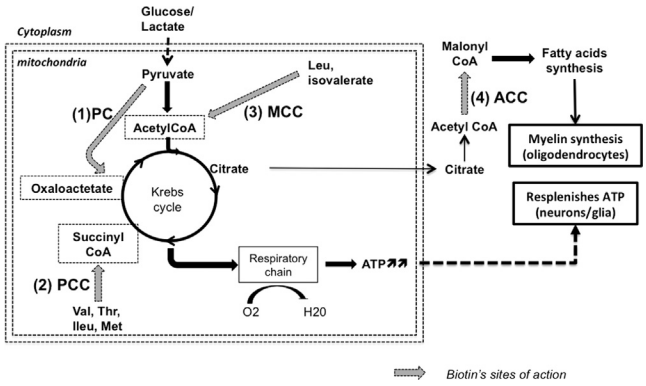

Fig. 4 Potential mechanisms of action of high doses of biotin. Targets of biotin are: (1) PC: pyruvate carboxylase, (2) PCC: propionyl CoA carboxylase, (3) MCC: methylcrotonyl CoA carboxylase, (4) ACC: acetylCoA carboxylases. Activation of PC, PCC, MCC may lead to increase ATP production in neurons (and astrocytes) whereas activation of ACC may lead to myelin synthesis by oligodendrocytes. Adapted from Tong, 2013 and from Rinholm et al. (2011) (see also text).

to the outer mitochondrial membrane (ACC2,Tong, 2013). PC, PCC and MCC are expressed in astrocytes and neurons (Hassel, 2000; Ballhausen et al., 2009) and are involved in the production of oxaloacetate, succinyl- CoA and acetyl CoA CoA that are key intermediates for the tricarboxylic acid (Krebs) cycle which plays a central role in neuronal energy production (Fig. 4). Activation of the Krebs cycle by very high doses of biotin may therefore increase the energy production in axons, thus avoiding the "virtual hypoxia phenomenon". On the other hand, ACC1 (and ACC2) is involved in the synthesis of malonyl CoA from acetyl CoA and citrate (Fig. 4). The synthesis of Malonyl CoA represents the rate-limiting and committed step of long-chain fatty acid biosynthesis. In the nervous system, ACC immunoreactivity is high in oligodendrocytes (Tansey et al., 1988), and its activity is detected in purified myelin (Chakraborty and Ledeen, 2003), suggesting that ACC (either ACC1 or ACC2) might be a key regulator for myelin synthesis. Furthermore, studies in cell cultures have shown that lactate, the main energetic substrate in the central nervous system, is oxidized in the Krebs cycle to produce ATP in neurons, whereas oligodendrocytes use lactate in part to produce membrane lipids presumably for myelin (Sanchez-Abarca et al., 2001; Rinholm et al., 2011). Overall, high doses of biotin, could target the main metabolic processes related to progressive MS by (1) activating the Krebs cycle in demyelinated axons to increase energy production; (2) activating the Krebs cycle in oligodendrocytes to increase the production of citrate required for lipids synthesis and; (3) activating ACC1 and ACC2, the rate-limiting enzymes in the synthesis of long chain fatty acids required for myelin synthesis (Fig. 4).

The adequate daily intake of biotin in adults is 30 μg and the dose used in this study was 10,000 more. Oral biotin is completely absorbed, urinary excretion of biotin and its metabolites being similar after intravenous and oral administration (Wang et al., 2001). Biotin is transported across the blood–brain barrier by a saturable system; the apparent K_m being about 100 μmol/L, a value several orders of magnitude greater than the concentration of free biotin in plasma even after administration of very high doses (Zempleni and Mock, 1999; Spector and Mock, 1987). Accordingly, it is expected that high doses of biotin administered orally will reach the brain to be incorporated into apocarboxylases *in fine* (Spector and Mock, 1988).

5. Conclusion

These data suggest that high doses of biotin may impact disease's progression and improve clinical sequelae in primary and secondary progressive MS. They rely on a case reports series and need to be confirmed. Two multicentric double-blind placebo-controlled trials are currently underway.

Conflicts of interest

Frédéric Sedel is currently CEO and shareholder of medDay pharmaceuticals. Other authors have no conflict of interest related to this study. This study received no funding.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.msard.2015.01.005.

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