A pilot trial of deferiprone for neurodegeneration with brain iron accumulation

Giovanni Abbruzzese,[1] Giovanni Cossu,[2] Manuela Balocco,[3] Roberta Marchese,[1] Daniela Murgia,[2] Maurizio Melis,[2] Renzo Galanello,[4] Susanna Barella,[4] Gildo Matta,[5] Uberto Ruffinengo,[6] Ubaldo Bonuccelli,[7] and Gian Luca Forni,[3]

[1]Department of Neurosciences, University of Genoa; [2]Neurology Department, "G. Brotzu" General Hospital, Cagliari; [3]Centro della Microcitemia e Anemie Congenite-Haematology, Galliera Hospital, Genoa; [4]Ospedale Regionale Microcitemia, University of Cagliari, Cagliari; [5]Radiology Department "G. Brotzu" General Hospital, Cagliari; [6]Neuroradiology Unit, Galliera Hospital, Genoa; [7]Neuroscience Department, University of Pisa, Italy

ABSTRACT

Deferiprone was shown to reverse iron deposition in Friedreich's ataxia. This multi-center, unblinded, single-arm pilot study evaluated safety and efficacy of deferiprone for reducing cerebral iron accumulation in neurodegeneration with brain iron accumulation. Four patients with genetically-confirmed pantothenate kinase-associated neurodegeneration, and 2 with parkinsonism and focal dystonia, but inconclusive genetic tests, received 15 mg/kg deferiprone bid. Magnetic resonance imaging and neurological examinations were conducted at baseline, six and 12 months. Chelation treatment caused no apparent hematologic or neurological side effects. Magnetic resonance imaging revealed decreased iron accumulation in the globus pallidus of 2 patients (one with pantothenate kinase-associated neurodegeneration). Clinical rating scales and blinded video rating evaluations documented mild-to-moderate motor improvement in 3 patients (2 with pantothenate kinase-associated neurodegeneration). These results underline the safety and tolerability of deferiprone, and suggest that chelating treatment might be effective in improving neurological manifestations associated with iron accumulation. *(Clinicaltrials.gov Identifier: NTC00907283)*

Key words: deferiprone, iron overload, neurodegeneration with brain iron accumulation.

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Introduction

Quantities of iron in the brain increase with age, but its accumulation in specific regions is observed in the heterogeneous group of diseases marked by neurodegeneration with brain iron accumulation (NBIA).[1-3] A diagnosis of NBIA can be suspected when there is evidence of representative clinical features with prominent extrapyramidal movement disorders (dystonia, parkinsonism, choreoathetosis), intellectual deterioration, and a characteristic deposition of iron in the basal ganglia.[3-4] Magnetic resonance imaging (MRI) has enabled premortem diagnosis of this condition[5] and confirmatory molecular genetic testing can now be performed in many cases. NBIA mainly includes: pantothenate kinase-associated neurodegeneration (PKAN), associated with mutations in the pantothenate kinase-2 gene (*PANK2*); NBIA type 2, associated with mutations in the calcium-independent phospholipase A2 gene (*PLA2G6*); neuroferritinopathy (NFT), associated with mutations in the ferritin light chain gene (*FTL*); and aceruloplasminemia, associated with mutations in the ceruloplasmin gene (*CP*). Other subtypes of NBIA have also been identified.[3,4,6]

Although treatment of systemic iron overload has significantly improved in the past decade,[7] no established therapy exists for brain iron accumulation. This is partly because most available iron-chelating drugs cannot cross the blood–brain barrier, and because the quantity of iron that defines brain overload is lower than in systemic overload,[8] leading to higher risks of over-chelation toxicity. Deferiprone is an orally active bidentate iron chelator that was found to be particularly effective in chelation of intracellular iron and in the treatment of regional (e.g. cardiac) iron overloads. It is authorized for treatment of patients affected by thalassemia major in conditions of 'chelation not suitable for deferoxamine'. Deferiprone has physicochemical characteristics (low molecular weight, favorable octanol:water partition coefficient, neutral charge) that allow good permeability of mitochondrial walls and the blood–brain barrier.[9,10] In addition, in the setting of regional iron overload, it seems that deferiprone has iron-relocating and redistributing abilities enabling it to act as a reverse siderophore.[10,11]

Deferiprone (30 mg/kg/day) was used in 9 patients with Friedreich's ataxia (FA), evaluated using the International Cooperative Ataxia Rating Scale (ICARS) and brain MRI.[8] After six months of therapy, iron accumulation in dentate nuclei was reduced and there was significant improvement of neuropathy and ataxic gait. Similar results were reported using combined therapy with idebenone and deferiprone.[12] One case of putative NBIA was treated successfully at our center, resulting in the disappearance of choreic dyskinesias and the normalization of gait disturbances.[13]

Deferiprone, despite its possible side effects (gastrointestinal disturbances, transient increase of transaminases, and, especial-

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Correspondence: Gian Luca Forni, Haematology, Ospedale Galliera, Via Volta 6, 16128, Genova, Italy. Phone: international +39.010.5634557. Fax: international +39.010.57481056. Email: gianluca.forni@galliera.it

ly, agranulocytosis), currently represents the only possibility for removing and/or preventing iron accumulation in the brain. This was a multi-center, unblinded, single-arm pilot study, lasting one year, to evaluate the efficacy and safety of chelation therapy with deferiprone on cerebral iron accumulation in patients with a clinical diagnosis of NBIA.[4]

Design and Methods

Patients

Inclusion criteria were: patients over 18 years of age with neurological symptoms correlated with iron deposition in the basal ganglia as documented by MRI (T2* and T2 signal decrease in the basal ganglia), performed within six months of enrolment. Exclusion criteria were: inability to undergo MRI; renal insufficiency (creatinine >1.5 mg/dL); neoplasias, systemic cardiovascular, severe renal and hepatic diseases; known hypersensitivity to deferiprone; pregnancy and breastfeeding. Additional exclusion criteria were average alanine transaminase (ALT) levels over 300, variations in ALT or aspartate transaminase (AST) levels of 300% during the year prior to enrolment, and patients judged potentially unreliable and/or uncooperative with regard to study procedures.

The trial was approved by the E.O. Ospedali Galliera Ethics Committee and the local Ethics Committee at the Cagliari center. All participants gave written informed consent before entering the study.

Procedures

Patients received deferiprone solution (Apopharm, Toronto, ON, Canada) at 15 mg/kg po bid, prescribed and monitored by the Microcitemia Center in Genoa and the Microcitemia Center at the University of Cagliari Pediatric Clinic. This drug may be associated with significant side effects, such as neutropenia and agranulocytosis, that appear to result from an idiosyncratic mechanism, and are not correlated with dosage. To reduce the risk of possible drug interference with hematologic homeostasis, we used a lower dose than is normally administered in patients with systemic iron overload (75 mg/kg per day). During the trial, safety and tolerability of the drug was evaluated by measuring hemochrome (with leukocyte formula count) weekly, and iron serum, ferritin, transferrin, creatinine, blood urea nitrogen (BUN), AST, ALT, calcium, phosphorous, protein electrophoresis, total proteins, and zinc levels monthly.

Follow-up visits were performed at three, six and 12 months. The patients were video-taped and assessed by neurologists expert in movement disorders from the Department of Neurosciences (University of Genoa) and from the Unit of Neurology and Radiology (Brotzu Hospital of Cagliari). The Unified Parkinson's Disease Rating Scale (UPDRS/III – Motor Section), ICARS, and the Unified Dystonia Rating Scale (UDRS) were administered at baseline and during follow up. An independent neurologist made a blinded evaluation of the videotapes.

Magnetic resonance imaging

All patients underwent brain MRI at baseline and at six and 12 months follow up. The protocol included sequences for morphologic and quantitative assessment. Images were acquired on a 1.5 T magnetic resonance imaging (MRI) scanner (GE Signa HDx, Milwaukee, WI, USA) using an 8-channel phased array head coil.

For morphological and qualitative analysis, the protocol used in both the Genoa and Cagliari centers included multiplane DWI, T1, FLAIR, T2* and T2 sequences (T2 parameter: TR 9000, TE 126, FOV 24 cm, FA 90°, slice thickness: 4 mm, gap 1 mm matrix 256 x 256).

Two independent, experienced, blinded neuroradiologists reviewed the MRI scans to provide a qualitative evaluation based on appropriate analysis of *a priori* defined regions of interest (ROI).

Quantitative assessment of brain iron was performed with T2* relaxometry, using a gradient multi-echo T2* sequence (field of view 24 cm, 255 x 224 matrix, slice thickness 5 mm, gap 3 mm, TR: 400 ms, 10 echoes at TE from 3.5 ms to 54 ms, flip angle 50°, acquisition time 4 min) to acquire each axial brain slice at ten echo time. Quantitative T2* maps were calculated off-line using a custom made reconstruction algorithm (FuncTool v. 5.2.09, GE Medical Systems). It was possible to perform T2* in only 3 cases (patients 2, 3 and 4) due to interference from metallic dental devices present in 2 patients and movement artefacts in one patient. ROI were manually drawn by a single neuroradiologist (on T2* maps) in the globus pallidus, and signal intensity was measured at each echo time. Other ROI were drawn for reference in the dentate nuclei, caudate, and putamen.

To obtain the T2*value, a mono-exponential trend line was fitted to the equation $y=Ke^{-TE/T2^*}$, where K represents a constant, TE represents the echo time and y represents the image signal intensity. Two readers independently reviewed the data for qualitative evaluation.

Statistical analysis

Laboratory investigations were analyzed using parametric statistics. Non-parametric tests were used for clinical rating scales showing a non-normal distribution. Statistical significance was achieved on two-tailed P values < 0.05.

Results and Discussion

Eleven patients were enrolled: one withdrew consent after one month; one died in an accident two months after entering the study; and 9 are still in treatment. The 6 patients included in this primary analysis (3 males; age range 19-65 years, mean±SD = 36.5±17.1 years) were recruited between November 2008 and May 2009, and the one-year assessment was made in July 2010. Four patients were diagnosed with PKAN (evidence of *PANK2* mutations). The other 2 presented with parkinsonism associated with cranio-cervical dystonia, but genetic testing (*PANK2*, *FTL*, *CP*) was inconclusive.[14]

Patient 1 had moderately elevated serum ferritin levels and normal transferrin saturation, but no familiarities, alimentary disorders or signs and symptoms of organ damage. He presented with C282Y/H63D double heterozygotes for the HFE mutation, but hepatic iron overload was excluded by T2* MRI. Patient 3 had a microcytosis and was heterozygous for the *alpha-globin* gene locus anti *3.7 kb* arrangement. Patient 5 had serum ferritin levels at the upper limit of normal for his age and gender while his transferrin saturation levels were normal. Patients' main baseline clinical features are summarized in Tables 1, 2 and 3.

Deferiprone safety and tolerability

Treatment with deferiprone proved safe and well tolerated. Gender and age characterized the pre-treatment hematologic picture. Hyposideremic anemia was only seen in one female patient with concomitant hypermenorrhea. In this case, treatment with deferiprone was interrupted for 15 days and additional iron was prescribed. No other side effects were reported by the patients during the observation period. Laboratory investigations did not reveal any treatment-related abnormalities (Table 2).

Changes in magnetic resonance imaging

Blinded evaluation by 2 neuroradiologists showed agreement in the identification of reduced hypointensity in the globus pallidus (GP) of 2 patients (cases 3 and 5) at the 6- and 12-month visits.

Quantification of brain iron through T2* relaxometry was possible only in patients 2, 3 and 4 due to the presence of signal interferences from metallic oral devices in other patients. A quantitative assessment showed a significant increment in the T2* value, and hence reduction of the iron content of the GP of these patients (baseline values 24.6, 19.7, and 20.0, respectively, *vs*. 33.6, 28.4, and 25.0, respectively, at 12 months). No differences were found in the T2* values of regions of interest in other parts of the brain. These data are in agreement with previous qualitative results suggesting that deferiprone can reduce iron burden in the GP.

Clinical changes

Clinical rating scales indicated an improvement in motor symptoms in 3 patients (Table 3). Patient 1 showed a marked reduction of the UPDRS/III score at both six and 12 months, and patients 2 and 4 showed a mild reduction of UPDRS/III and ICARS scores at 12 months. No evident change was observed in the other patients, although a worsening trend was noticeable in patient 7. The mean UPDRS/III motor score was significantly reduced both at six (P=0.04) and at 12 months (P=0.05). Blinded evaluation of video-tapes paralleled the findings of the rating scales, with patients 1, 2 and 4 judged to be showing clinical improvement.

Iron accumulation within the brain is generally thought to cause neural damage due to the ability of labile forms of iron to induce oxidative stress.[15] Abnormal iron deposition in the basal ganglia can be observed in several neurodegen-

Table 1. Baseline characteristics of patients.

UPN	Sex	Age	Disease duration (yrs)	Clinical picture	Diagnosis	MRI
1	M	49	4	Parkinsonism Cervical dystonia	NBIA	T2* hypointensities in GP, PUT, MES
2	F	26	3	Multifocal dystonia	PKAN (*Pank2* +)	T2* hypointensities in GP (bilateral) – 'tiger eye' sign
3	F	29	13	Multifocal dystonia Parkinsonism	PKAN (*Pank2* +)	T2* hypointensities in GP (bilateral) – 'tiger eye' sign
4	M	31	11	Multifocal dystonia Parkinsonism	PKAN (*Pank2* +)	T2* hypointensities in GP (bilateral) – 'tiger eye' sign
5	M	65	9	Parkinsonism Cranial dystonia	NBIA	T2* hypointensities in GP (bilateral)
7	F	19	5	Multifocal dystonia Parkinsonism	PKAN (*Pank2* +)	T2* hypointensities in GP (bilateral) – 'tiger eye' sign

UPN: unique personal number; GP: globus pallidus; PUT: putamen; MES: mesencephalon.

Table 2. Laboratory results.

	Basal				6 months				12 months			
UPN	TS%	Ferritin	Hb	MCV	TS%	Ferritin	Hb	MCV	TS%	Ferritin	Hb	MCV
1	47.0	450.0	16.7	89.0	44.0	430.0	15.9	89.0	44.0	420.0	16.1	83.0
2	33.7	25.2	13.0	91.1	8.0	5.2	11.1	87.3	14.0	3.9	11.2	84.0
3	20.9	47.2	12.6	76.4	23.0	16.4	11.7	75.5	11.0	10.5	12.3	74.9
4	31.0	65.0	13.8	82.0	30.0	60.0	13.6	81.0	31.0	62.0	13.5	81.0
5	22.0	398.0	16.3	85.0	25.0	388.0	14.4	83.0	30.0	236.0	14.4	85.0
7	21.0	72.0	12.7	82.0	27.0	23.0	12.7	81.0	10.0	12.0	12.1	80.0

UPN: unique personal number; Hb: hemoglobin; MCV: mean corpuscolar volume; TS%: percentage transferrin iron saturation.

Table 3. Clinical evaluation of patients.

UPN	UPDRS motor score			UDRS global score			ICARS score			Blinded Video Rating	
	Basal	6 mo	12 mo	Basal	6 mo	12 mo	Basal	6 mo	12 mo	6 mo	12 mo
1	21	9	7	4	4	4	na	na	na	Improved slightly	Improved moderately
2	13	10	9	5	5	5	9	7	5	Improved slightly	Improved slightly
3	47	44	43	21	21	21	28	26	22	Unchanged	Unchanged
4	29	24	21	13	13	13	16	13	9	Improved moderately	Improved moderately
5	37	32	36	16	16	20	na	na	na	Unchanged	Unchanged
7	31	31	34	16	18	24	na	na	na	Unchanged	Unchanged

UPN: unique personal number; UPDRS: unified Parkinson's disease rating scale; UDRS: unified dystonia rating scale; ICARS: international cooperative ataxia rating scale; mo: months; na: not applicable.

erative disorders including the NBIA group. Iron misregulation, which results in intracerebral accumulation and consequent neurodegeneration, is commonly considered an important clue to the etiology of these illnesses. The use of chelating agents has achieved significant success in the treatment of systemic iron overload;[7] nevertheless, the possibility of chelating iron accumulated in specific brain regions still remains an open question and no effective treatment for NBIA is currently available.

Preliminary data from this phase II pilot study show that treatment with deferiprone is safe and well tolerated. No serious adverse events were observed after one year of treatment, although we cannot rule out the possibility that this is related to the reduced dosage adopted. Qualitative and quantitative evaluation of MRI showed that deferiprone was able to reduce brain iron accumulation in some patients, confirming previous observations in FA.[8,11] A significant change in the multi-echo T2–T2* signal was documented in the GP of 3 patients, suggesting that the drug is apparently able to partially remove chelatable iron in different brain regions. This observation confirms the reduction in GP iron content, as assessed by T2* relaxometry, observed by Zorzi *et al.* in 9 subjects with genetically-confirmed PKAN treated with deferiprone (25 mg/kg/day).[16]

Three subjects (including 2 with PKAN) showed mild-to-moderate improvement in motor symptoms, documented by changes in clinical rating scales and blinded assessment of video tapes. No significant change was observed in the other 3 patients. Although all the patients enrolled in the study fulfilled the clinical criteria for the diagnosis of NBIA, their clinical profiles were heterogeneous, including large variations in age at onset, disease duration and severity. PKAN patients in the study by Zorzi *et al.*[16] did not show any clinical improvement despite the effective reduction in brain iron accumulation.[16] However, these patients were treated for a shorter period (six months) and most of them were severely affected and had been ill for a long period of time. A possible explanation of the clinical improvement in our patients might be that the iron-related neurodegenerative process was still partially reversible in patients with relatively recent onset (patients 1 and 2) or with a slower rate of progression of the disease, suggesting that the efficacy of the drug might be correlated to disease stage.

Clinical progression in NBIA cases, especially in the adult-onset subtypes, is uncertain and largely variable, thus observation at one-year is certainly insufficient to draw any conclusions. The subsequent follow up of our cases might help to clarify whether iron chelation can slow down or halt the progression of the disease. Also, the quantitative assessment of iron deposition in specific brain regions needs to be standardized and the relationship between the modifications of clinical profiles and MRI patterns clarified. For instance, it has been demonstrated that after intensive chelation in patients with cardiac iron overload, cardiac function is soon significantly restored, although it takes longer to improve the values of iron overload as measured by MRI T2* . In keeping with Zorzi *et al.*[16] we did not observe a significant correlation between clinical and radiological findings.

The preliminary data from our pilot study underline the safety and tolerability of deferiprone as a chelator agent for intra- and extraneuronal iron accumulation. The clinical benefit observed in some of our patients suggests that treatment with deferiprone may be associated with an improvement in neurological manifestations linked with iron accumulation and neurodegeneration; however, these results need to be confirmed in a larger randomized study with a prolonged observation period.

Authorship and Disclosures

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