Hormones and Behavior 63 (2013) 331–344

Contents lists available at SciVerse ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Review

Growth hormone (GH) and brain trauma

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ARTICLE INFO

Available online 1 March 2012

Keywords:
TBI
GH
IGF-1
Cognitive disorders
Neurogenic dysphagia
Hemi/tetraplegia
Amaurosis
Physiotherapy
Visual stimulation
Remyelination

ABSTRACT

This article is part of a Special Issue "Hormones & Neurotrauma".

Growth hormone (GH) is a pleiotropic hormone with known neurotrophic effects. We aimed to study whether GH administration might be useful together with rehabilitation in the recovery of TBI patients.
13 TBI patients (8 M, 5 F; age: 6–53 years old) were studied. Time after TBI: 2.5 months to 11 years; 5 patients showed acquired GH-deficiency (GHD). Disabilities observed: cognitive disorders; motor plegias; neurogenic dysphagia (n = 5), vegetative coma (n = 2) and amaurosis (n = 1). All but one TBI patient followed intense rehabilitation for years. Treatment consisted of GH administration (maximal dose 1 mg/day, 5 days/week, resting 15-days every 2-months, until a maximum of 8 months) and clinical rehabilitation according to the individual needs (3–4 h/day, 5 days/week, during 6–12 months). Informed consent was obtained before commencing GH administration.
GH significantly increased plasma IGF-1 values ($ng.mL^{-1}$) in both GHD and no GHD patients, being then similar between both groups (GHD: 275.6 ± 35.6 [$p<0.01$ vs. baseline], no GHD: 270.2 ± 64 [$p<0.05$ vs. baseline]).
In all the cases clear significant improvements were observed during and at the end of the combined treatment. Cognitive improvements appeared earlier and were more important than motor improvements. Swallowing improved significantly in all TBI patients with neurogenic dysphagia (2 of them in a vegetative state). Visual performance was ameliorated in the patient with amaurosis. No undesirable side-effects were observed.
Our data indicate that GH can be combined with rehabilitation for improving disabilities in TBI patients, regardless of whether or not they are GHD.

Contents

Introduction

Traumatic brain injury (TBI), represents one of the largest health problems in developed countries, both in terms of the number of deaths caused and the high number of people left suffering from some sort of functional and cognitive disability as a result of the sequelae caused by the damage that occurred in the brain (Hyder et al., 2007).

The multiple functional and social impairments caused by the brain injury and the motor impairments affecting memory, speech, deglutition, walking or behavior require a multi-disciplinary treatment approach from the critical–acute stage, calling for the highest level of specialization until the patient can readjust back into the community. There is consensus among specialists regarding the damaged

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doi:10.1016/j.yhbeh.2012.02.022

brain's ability to recover part of its functions spontaneously, a process that may take several months or even years. Experts also agree on the need for early neurorehabilitation to improve these natural mechanisms and achieve the best possible functional and social recovery. However, access to rehabilitation facilities specialized in brain injury is marked by the shortage of public or private resources, with a huge difference in the availability of such facilities among the different countries.

It is known that when brain tissue is damaged, cells usually continue to die even when the initial stimulus has stopped. That is, the destiny of neurons in a damaged brain depends on a fragile equilibrium between pro-survival and pro-death signals. Pro-death signals can be triggered by oxidative damage or because of incorrect neurotransmitter signals such as enhanced glutamate release (excitotoxicity). From these concepts it is logical to assume that knowing how to act precociously on both kinds of signals would allow one to prevent the severity of the damage after TBI.

Two questions arise then: how to act precociously in terms of pharmacological repair and, how to help in the recovery of TBI patients in which the damage occurred some time ago.

Classically, it is thought that recovery from a brain injury occurs through the reinforcement of the mechanisms of neural plasticity. Establishing new synaptic connections between surviving neurons would partially allow the recovery of lost functions. There are evidences that environmental enrichment induces epigenetic changes that facilitate synaptogenesis and memory in models of brain plasticity (Johansson, 2011). However, today we know that apart from brain plasticity, the development of any brain injury quickly leads to enhanced proliferation of neural stem cells. From the damaged cerebral areas a number of cytokines would be released for activating the migration and differentiation of newborn cells. This is a physiological mechanism that the brain uses in an attempt to repair any damaged area, but it is usually not strong enough for achieving a complete repair.

That is, two different mechanisms are involved in trying to repair brain damage: 1) quick proliferation of neural precursors, and 2) development of neural plasticity.

Both are independent but complementary of each other. Neural stem cell proliferation and brain plasticity require the intervention of neurotrophic factors. These concepts are schematized in Fig. 1.

The paradigm about a central nervous system unable to replace the daily loss of neurons began to be questioned in the mid-60s. We currently know that constitutive neurogenesis takes place in some particular niches of adult mammalian brain. Following cerebral injuries, neurogenesis increases and the newly formed neurons are able to repopulate damaged areas (Parent, 2003; Romanko et al., 2004; Zhang et al., 2004). Recent studies indicate that apart from the already considered classical neurogenic niches present in the adult mammal brain (subventricular zone and subgranular zone of the hippocampal dentate gyrus) adult neurogenesis can also occur in other brain areas. It is thought that in the adult mammal brain physiological anti-neurogenic influences can be removed in pathological conditions or after any specific injury. This has been recently demonstrated in a model of unilateral vestibular neurectomy that mimics human pathology in adult cats (Dutheil et al., 2011). Moreover, following brain injury, glia outside known neurogenic niches acquires or reactivates stem cell potential as part of reactive gliosis (Robel et al., 2011). A comparison of molecular pathways activated after injury with those involved in the normal neural stem cell niches highlights strategies that could overcome the inhibition of neurogenesis outside the stem cell niche and instruct parenchymal glia towards a neurogenic fate. This new view on reactive glia therefore suggests a widespread endogenous source of cells with stem cell potential, which might potentially be harnessed for local repair strategies (Robel et al., 2011). Therefore the knowledge about how adult neurogenesis is regulated may provide adequate therapeutic tools for trying to repair the brain after an injury.

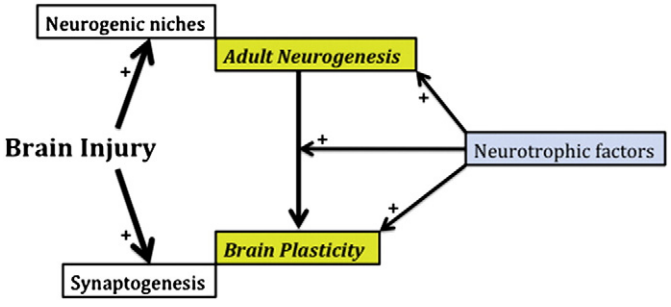

Fig. 1. Brain injury leads to a prompt stimulation of the proliferation of neural precursors in brain neurogenic niches resulting in adult neurogenesis. Later, neurorehabilitation and rich environment produce adaptative responses occurring as a consequence of increased synaptogenesis: brain plasticity. Adult neurogenesis contributes to brain plasticity. Both processes require the intervention of neurotrophic factors. + indicates stimulation or facilitation.

Several preclinical studies indicate that stem cell therapies are promising for treating TBI (for a detailed review see Heile and Brinker, 2011). Among them, mesenchymal stem cells (MSCs) have been reported to induce neuroprotective and regenerative effects following cerebral ischemia and TBI (Longhi et al., 2005; Bliss et al., 2007). Direct intracerebral administration of MSCs has been suggested to be effective for neural repair in rats (Chen et al., 2001). These cells have been shown to act mainly through the release of neurotrophic and immunomodulatory peptides (Ohtaki et al., 2008), including insulin-like growth hormone factor 1 (IGF-1), rather than through cell replacement or direct cell-to-cell contact (Samowska et al., 2009). That is, MSCs would represent a source of trophic support facilitating endogenous repair by stimulating neurogenesis, angiogenesis and synaptogenesis (Savitz, 2009). MSC therapies are being investigated in phase I clinical trials in stroke patients, but preliminary studies in rats showed that their effectiveness is limited to a therapeutic time window up to 72 h after stroke (Yang et al., 2011), decreasing after it. Most likely this is due to the death of implanted MSCs. Trophic factor secretion is postulated as a primary or secondary mechanism of action for many transplanted cells, however, there is little evidence to support trophin production by transplanted cells in situ.

A current clinical trial (http://www.clinicaltrials.gov/ct2/show/NCT01298830?term=glp-1+AND+stroke&rank=1. Accessed April 14. 2011) is investigating the effects of intracerebral implants of microencapsulated allogenic hMSCs delivering glucagon-like peptide-1 (GLP-1), a known neuroprotective and neurotrophic peptide (Gilman et al., 2003; Perry et al., 2003; During et al., 2003). Preliminary results from the ongoing clinical trial show that this procedure might be an effective treatment for TBI patients (Heile and Brinker, 2011), but again the short half life of implanted MSCs seems to restrict this therapy to be used in the acute phase of TBI.

In all, these data reflect that currently treatments have not been described, apart from kynesitherapy, for trying to recover patients once the acute phase of TBI has resolved and the patient is discharged from the hospital. However, these data will allow us to justify why we tested growth hormone (GH) administration for trying to improve the recovery of TBI patients.

The hypothesis that GH and insulin-like growth factor 1 (IGF-I) play a role on brain repair after an injury has been postulated years ago (Scheepens et al., 2000). However, most of the studies carried out basically analyzed the role of IGF-1 (Laron and Klinger, 1994; Hatton et al., 1997; Aberg et al., 2000; Aberg et al., 2007). Despite these, a number of data support a role for GH itself in neurogenesis. GH receptor is expressed in regions of the brain in which neurogenesis occurs during embryonic brain development (García-Aragón et al., 1992; Turnley et al., 2002) and in neurogenic regions of the

postnatal rat brain (Lobie et al., 1993). Growth hormone itself is also found in cells of the ventricular zone during embryonic neurogenesis (Turnley et al., 2002), and is produced endogenously within the postnatal hippocampus (Donahue et al., 2002; Donahue et al., 2006; Sun et al., 2005a; Sun et al., 2005b). Interestingly, GH gene expression within the hippocampus is increased by some factors known to increase neurogenesis (Parent, 2003), including learning (Donahue et al., 2002) and estrogen (Donahue et al., 2006).

The role of GH in the hippocampal dentate gyrus has been related to neuronal survival rather than generation, as altered hippocampal GH levels have no effect on cell proliferation but do affect the survival of immature neurons (Sun et al., 2005b; Sun and Bartke, 2007; Lichtenwalner et al., 2006). Nevertheless, studies of the effects of GH on embryonic rat cerebral cortical (Ajo et al., 2003) and hippocampal (Byts et al., 2008) neuronal cultures found that it induces the proliferation and differentiation of these cells. More recently, Pathipati et al. (2011) demonstrated that exogenously applied GH and PRL promote the proliferation and migration of neural stem cells derived from fetal human forebrains in the absence of EGF or bFGF. This agrees with previous preclinical data from our group and others demonstrating that exogenous GH administration promotes the proliferation of hippocampal neural precursors after brain injury induced by kainate administration (Devesa et al., 2011a) and in a number of zones in the intact adult rat brain (David Aberg et al., 2010). In this regard it is important to remark that GH easily crosses the blood–brain-barrier (BBB) (Pan et al., 2005).

According to these evidences it is likely that GH may facilitate the proliferation, differentiation and survival of new neurons in response to brain injury. However, to date, only few studies in humans explore such a possibility. While these studies indicate a positive effect for GH treatment together with specific neurorehabilitation, both in children with cerebral palsy (Reimunde et al., 2010; Devesa et al., 2011a) and in TBI patients (High et al., 2010; Reimunde et al., 2011), or in a patient suffering from a neurogenic dysphagia after oncological brain surgery (Devesa et al., 2009), all patients in these studies had GH deficiency (GHD) most likely occurring as a consequence of their brain damage.

No studies have been carried out for analyzing the possibility that GH administration, together with rehabilitation, may be useful for the recovery of TBI patients without GHD. However, given the preclinical evidences and since GH induces the expression and/or the release of a number of factors with known neurotrophic properties (Fig. 2) it seems to be logical to assume that the hormone may also facilitate the response to rehabilitation therapies in TBI patients without GHD.

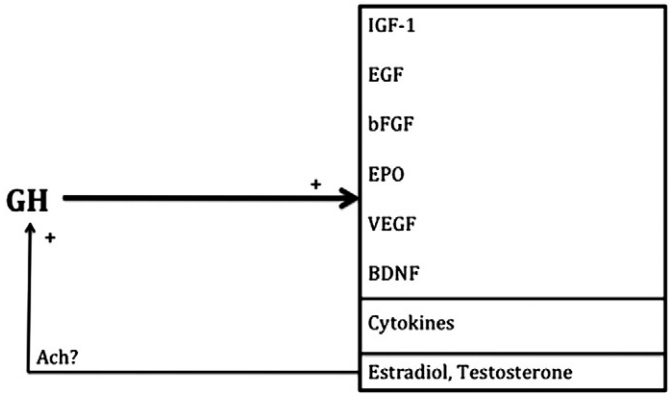

Fig. 2. GH induces the expression of a number of neurotrophic factors: IGF-1, EGF (epidermal growth factor) and its receptor, bFGF (basic fibroblast growth factor), EPO (erythropoietin), VEGF (vascular endothelial growth factor), BDNF (brain-derived neurotrophic factor; not demonstrated yet whether its expression is directly or indirectly induced by GH). These are pivotal factors for neurogenesis and angiogenesis. In addition, GH stimulates the release of a number of cytokines playing a role in the brain response to an injury. At the gonadal level the hormone facilitates the synthesis of estradiol and testosterone; these, in turn, potentiate pituitary GH synthesis and release (an effect most likely mediated by Ach). In all, while GH is capable of directly acting on brain neural precursor proliferation, its effects on this process can be potentiated by the neurotrophic factors induced by the hormone.

In this review we describe several cases of TBI patients, with and without acquired GHD, treated with GH and specific rehabilitation; they were selected because of the complexity of their TBI and/or the time elapsed since TBI occurred in them. The heterogeneity of the cases (age, type of brain injuries, time elapsed since injuries occurred, etc.) does not allow us to perform a statistical analysis of results obtained, excepting for changes in plasma IGF-1 values; therefore each case is presented as an individual case report.

Material and methods

Studies were conducted with the understanding and written consent of each patient or their legal representatives, and in compliance with national legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki).

All the patients had suffered a TBI at least in the previous 12 years and all had received intensive rehabilitation for their disabilities. Brain injuries mainly occurred because of frontal contusions. Medical records after TBIs were provided by patient's relatives.

After admission at Medical Center “Proyecto Foltra” a clinical history was performed and specific cognitive and motor tests showed the type and degree of disability of each patient. These tests were repeated at 3-month intervals, excepting when clear improvements were observed between these 3-month periods; in this case the time between assessments was reduced.

The existence of GHD was established or discarded according to the GH response to the GHRH–arginine test (GHD was considered to exist when peak plasma GH values were below 7 $ng.mL^{-1}$ in response to this test). GH plasma levels were measured by a solid-phase, two-site chemiluminiscent immunometric assay (Inmulite 2000, Siemens). Other pituitary hormone deficiencies were discarded by chemiluminiscent immunometric assays. IGF-1 and IGFBP-3 plasma levels were measured by a solid-phase, enzyme-labeled chemiluminiscent immunometric assay (Inmulite 2000, Siemens).

Before commencing GH treatment and at 2-month intervals after it routine blood analyses (hematology and chemistry) were carried out (Coulter HmX, Beckman and AU400, Beckman, respectively). Plasma TSH and fT4 and tumoral markers (PSA and CEA, in males; and CEA, CA125, CA15.3 and CA19.9 in females) were measured at similar time intervals.

Exclusion criteria for commencing GH treatment were: diabetes, hypothyroidism or hypocortisolism, cranial hypertension, papilledema, pregnancy or the existence of a malignant activity in the previous 5 years.

GH doses (rhGH; Omnitrope, Sandoz; or Nutropin, Ipsen) started with 0.2 mg/day for 15 days, then they were increased to 0.5 mg/day for another 15 days and then to 0.8–1 mg/day (depending on the age, weight and plasma values of IGF-1; we tried that plasma IGF-1 was not higher than 2SD over the mean for the age of the patient) until the end of treatment. GH was administered at 10:00 am, 5 days/week. After the first 2 months of treatment a resting period of 15 days was scheduled. After it, GH was administered again at the maximal dose scheduled for each patient following the same protocol procedure (2 months receiving the hormone–15 days without it) for a maximum of 8 months (regardless of whether rehabilitation would continue or not).

All patients received daily rehabilitation according to the specific individual needs as indicated by the initial assessment (physiotherapy, speech therapy, cognitive rehabilitation, visual stimulation, occupational therapy, etc.). Each of these therapies was carried out 1 h per day, with the exception of visual stimulation. For visual stimulation, 2 sessions of 15 min of duration were carried out daily.

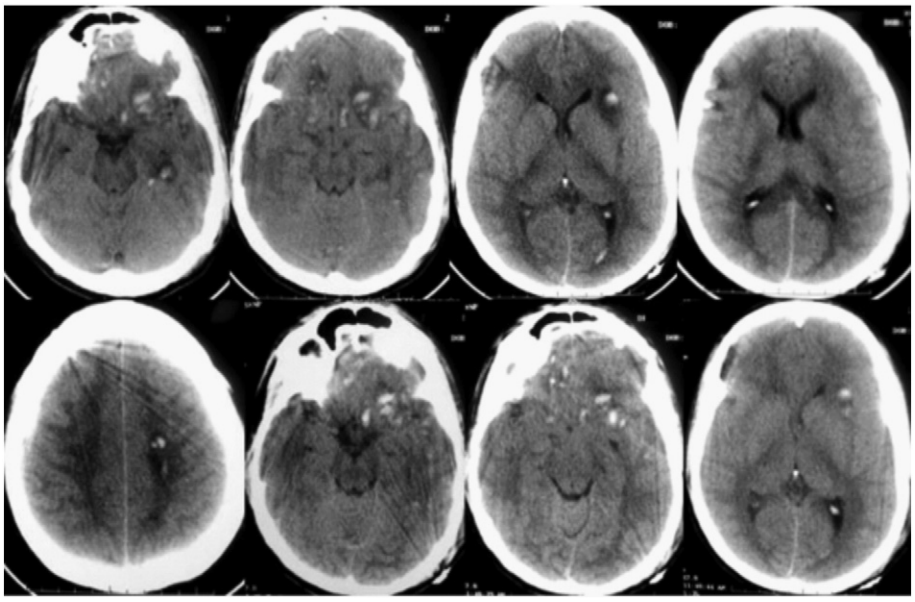

Fig. 3. Case 1. Brain CAT scan performed 12 h after TBI. Images show hemorrhagic contusions in brain frontal lobes and left temporal lobe. Blood in left Vc. Subarachnoidea hemorrhage. Inferior maxillar fracture.

Visual stimulation was performed by using a tachistoscope (repetitive white light flashes, 100–150 ms, carried out in 10 phases lasting 1 min each, 80 flashes/min; XLTEC™ photic stimulator, Model XLPS-1F). That is, each treatment session consisted of 10 phases and each phase of stimulation consisted of 10 sets of stimulation in which flashes of white light are released during 1 s at a frequency of 6–8 Hz. Between each series of stimulation there is a pause of 5 s. Each phase of stimulation performed 80 flashes per minute. Between each phase of stimulation a pause of 2 min in absolute darkness was applied. Stimulation was performed in a dark isolated room.

Plasma IGF-1 values before and after GH treatment were compared by Wilcoxon signed rank test. Statistical significance of the data was established at $p<0.05$.

None of the patients withdrew medication previously prescribed at discharge from each preceeding hospital or medical doctors treating them before admission in our medical center.

Case report

Case 1. Male, 22 years old. High-speed motor vehicle accident with a severe TBI (Glasgow Coma Scale, GCS, 6), remaining in coma for 15 days. Brain CAT scan study performed 12-h after admission (Fig. 3) revealed the existence of a subarachnoid hemorrhage, multiple punctate hemorrhagic contusions in brain frontal lobes and left temporal lobe, presence of blood in left ventricle, and fracture of lower jaw or mandible. A subsequent MRI study demonstrated the existence of brain stem injury not observed in CAT scan. The patient was discharged from the hospital and commenced with GH therapy and rehabilitation 2.5 months after TBI.

First studies revealed a very poor physical and cognitive condition, right leg monoplegia and right hand tremor, important cognitive (temporo-spatial confusion, loss of recent memory) and mood impairments, left facial and oropharynx hemiparalysis and a decreased sensitivity in left hemipharynx. The patient showed dysphonia and neurogenic dysphagia, and had to be fed by nasogastric tube. Blood exams demonstrated GHD, but no other pituitary deficiencies. The patient was scheduled for GH treatment (maximal GH dose 1 mg/day) and rehabilitation consisting of physical therapy, speech therapy and psychomotor therapy.

One month after the onset of the treatment signs of left tongue hemiparalysis (Fig. 4A) had disappeared and tongue mobility had been completely recovered (Fig. 4B). Oropharyngeal function was completely recovered (assessed by both phonation and videolaryngoscopy, Figs. 5A and B) and, therefore, the nasogastric tube was removed. The functional outcome assessment of swallowing score (FOAMS) increased from 1 to 6 after 2 months of treatment, and to 7 after 6 months (Table 1). Motor and cognitive deficits, fully disappeared after 8 months of treatment, and the patient reached a total functional independence for day life activities. Plasma levels of IGF-1 increased from 54 $ng.mL^{-1}$ at admission to 321 $ng.mL^{-1}$ at discharge.

Case 2. Female, 21 years old. High-speed motor vehicle accident at age 9 resulting in a severe TBI, (GCS 6) remaining in coma for 9 months. Brain CAT revealed the existence of subarachnoid hemorrhage and massive brain edema. Cranial hypertension was also observed at that time. A second CAT scan performed 3-months post-injury showed important cortico-subcortical atrophy, bilateral fronto-parietal hygroma and porencephaly in temporo-occipital areas (Fig. 6A). A CAT scan performed 3 years later demonstrated lack of parenchyma in frontal, parietal, temporal and occipital lobes together with gliosis areas (Figs. 6B and C).

At admission in our center, 11 years after her TBI, clinical assessments showed right spastic hemiplegia, and severe cognitive impairment with aphasia, loss of memory and lack of attention, and comprehension. Bilateral amaurosis with anisocoria and bilateral mydriasis were also present (Table 1). Evoked visual potentials performed at this time indicated bilateral absence of any organized neural transmission from the retina (Fig. 7A). No pituitary hormonal deficits were found. The patient was scheduled for GH treatment (maximal GH dose 1 mg/day) and rehabilitation consisting of physical therapy, psychomotor stimulation, speech therapy, occupational therapy and visual stimulation.

Nine months after the onset of the therapy, the patient was able to detect moving objects (100% score) and bright lights (95% score for vertical movements, 90% score for horizontal movements and 60% score for circular movements). She was also able to detect some static objects. Visual functionality was increased from below level I to level II, according to the scale of Huo for visual functionality (Huo et al., 1999). Pupilar reactivity to light was also improved, and evoked visual

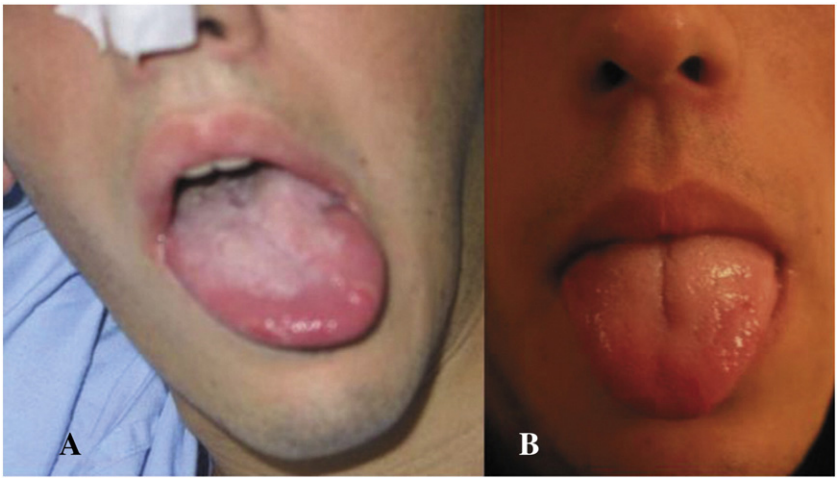

Fig. 4. Case 1. As shown in A there was a left tongue hemiparalysis before commencing GH treatment. Normal tongue mobility was completely recovered 1 month after GH treatment commenced (B).

potentials detected the N75 wave in left eye (Fig. 7B). PET revealed the existence of metabolic activity in the left occipital cortex at the level of cuneus/calcarine fissure (Fig. 8). Cognitive and motor affectations were also improved (Table 1), mainly those regarding memory, attention, orientation and expressive language. No side effects were observed during GH treatment. Plasma IGF-1 values were 317 ng.mL^{-1} before starting GH administration and 389 ng.mL^{-1} after finishing it.

Case 3. Male, 53 years old. High-speed motor vehicle accident at age 47 resulting in severe TBI (GCS 4). Brain CAT scan showed a left fronto-temporal petechial hemorrhagic contusion and a right parieto-temporal non hemorrhagic contusion. Electroencephalogram (EEG) records carried out during the first 2 months after injury showed diffuse alteration of brain bioelectrical activity with diffuse cortico-subcortical involvement, dysmetabolic focus with right parieto-temporal monomorphic delta waves and left fronto-parietal irritative focus. At hospital discharge, 1 year after admission, the patient showed neurologic sequelae that included paresis of right cranial nerve pair III, left brachial plexus injury with hemiplegia and hypoesthesia. The patient had no motor function in his left upper limb and coordination of fine motor tasks was impaired in his right hand. In addition, severe cognitive disorders existed, that showed a slight improvement after 6 years of intensive daily rehabilitation. Tonic–clonic seizures appear 2.5 years after hospital discharge.

At admission in our center a brain CAT scan showed areas of post-traumatic porencephaly. Tonic–clonic seizures were under pharmacologic control. No pituitary hormone deficiencies existed. Clinical assessment confirmed the existence of severe cognitive disorders with some mood abnormalities, and the persistence of paresis cranial nerve pair III and hemiplegia and hypoesthesia in his left upper limb. However, motor functionality had worsened since hospital discharge, with the appearance of ataxia, impaired motor coordination and standing balance and lower limb, so the patient needed the assistance of a person to walk and supervision in standing up. Impairments in eye movements and mild dysarthria were also present at this time. The patient was scheduled for GH treatment (maximal GH dose 0.8 mg/day) and rehabilitation consisting of physical therapy, speech therapy, psychomotor stimulation and occupational therapy.

After 6 months of treatment an important improvement of the motor impairment was observed, which included amelioration of left hemibody motor control, ataxia, motor coordination, standing balance, dystonia and dysarthria. At this time, the patient was capable of standing up without supervision or aid, and of walking with supervision or minimal assistance or using a technical aid. Coordination of upper limbs and fine and manipulative movements were also enhanced; and cognitive deficits improved from severe to moderate. All these changes are summarized in Table 1. Results of the routine blood test were normal, and plasma IGF-I (pre-treatment: 212 ng.mL^{-1}) remained in normal values at the end of the 6-month treatment (186 ng mL^{-1}). No adverse side effects were recorded. This patient is still under treatment.

Case 4. Female, 35 years old. High-speed motor vehicle accident at age 28 resulting in severe TBI (GCS 3). No other medical records were provided.

At admission, the patient showed spastic tetraparesis, anarthria, neurogenic dysphagia and a vegetative state. She had to be fed with a nasogastric tube. A brain CAT scan performed shortly before admission, showed a large area of encephalomalacia and post-traumatic gliosis affecting the entire left hemisphere and right frontal lobe, midbrain volume loss and important ventriculomegaly. Blood tests demonstrated GHD and increased levels of plasma gonadotrophins. Since

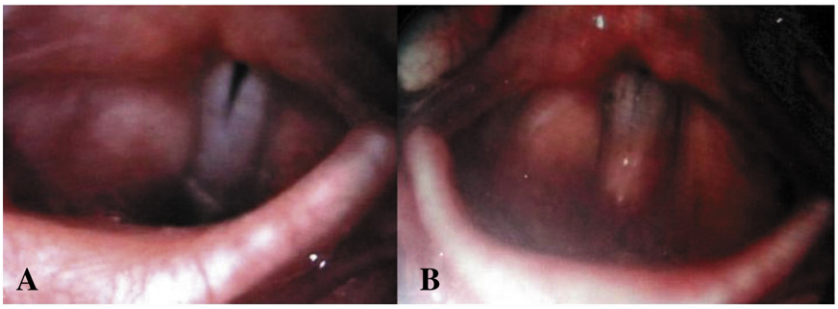

Fig. 5. Case 1. Videolaryngoscopy exam carried out before commencing GH treatment (A) and 1 month after it (B). As images show left oropharyngeal structures and vocal cord hemiparalysis had been completely recovered after 1 month under GH treatment and speech therapy.

Table 1
Main clinical assessments performed in adult patients.

Case/time	GHD	Dysphagia		Cognitive assessments				Motor assessments				Functional assessment	
		FOAMS		WAIS (IQT)		MMSE		FAC		Tinetti		MBI	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1/2.5 m	+	1	7	62	118	10	35	1	5	5	26	40	100
2/11 y	−	NP	NP	NP	NP	NP	NP	3	5	15	24	50	80
3/7 y	−	NP	NP	NP	NP	14	21	0	2	6	16	40	65
4/7 y	+	1	4	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
5/3 y	−	NP	NP	63	84	12	26	1	3	8	16	40	75
6/11 y	−	NP	NP	66	79	21	25	1	4	8	20	55	70
7/7 y	+	1	4	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
8/1 y	−	NP	NP	55	85	14	24	0	1	NP	NP	40	60
9/14 m	+	1	5	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
10/3 y	+	2	6	58	95	15	30	0	2	5	12	40	55
11/1.5 y	−	NP	NP	50	71	15	22	0	1	NP	NP	40	55
12/8 y	−	NP	NP	75	98	NP	NP	3	5	14	24	74	90

Notes. Case/time: patient number in case report/time elapsed since TBI and admission (m = months; y = years). FAC: functional ambulatory category; FOAMS: Functional Outcome Assessment Measure of Swallowing; MBI: Modified Barthel Index; MMSE: mini mental state examination; Tinetti: balance and gait tests (total scores); WAIS: Wechsler Adult Intelligence Scale (IQT: Total intelligence quotient); + = GHD existed; − = no GHD was detected; pre: pre-treatment; post: post-treatment; NP: assessment not performed.

amenorrhea appeared few years after her TBI, we assumed that a precocious menopause existed as a consequence of her TBI or her chronically ill condition. The patient was scheduled for GH treatment (maximal GH dose 1 mg/day) and rehabilitation consisting of physical therapy, speech therapy and psychomotor stimulation.

Twelve months after the onset of the treatment spasticity decreased and the patient was able to voluntarily move her arms. The degree of consciousness increased to a minimally conscious state with no evolution in speech. The FOAMS increased from 1 to 4, though the nasogastric tube could not be removed. Plasma IGF-1 values increased from 36 $ng.mL^{-1}$ to 286 $ng.mL^{-1}$ at the end of GH treatment period. No undesirable side-effects were observed during GH treatment.

Case 5. Male, 35 years old. Frontal impact after parachute failure at age 32 resulting in severe TBI (GCS 3) remaining in coma for 6 months. No other medical records were provided.

At admission, the patient showed spastic right hemiplegia, severe cognitive impairment with an important loss of recent memory, mood disorders, dysarthria, and right eye blindness. A CAT scan performed shortly before admission revealed a severe cortical atrophy, frontal lobe injuries with areas of malacia, and ventriculomegaly. No pituitary hormonal deficits were found. The patient was scheduled for GH treatment (maximal GH dose 1 mg/day) and rehabilitation consisting of physical therapy, speech therapy, psychomotor stimulation, occupational therapy and visual stimulation.

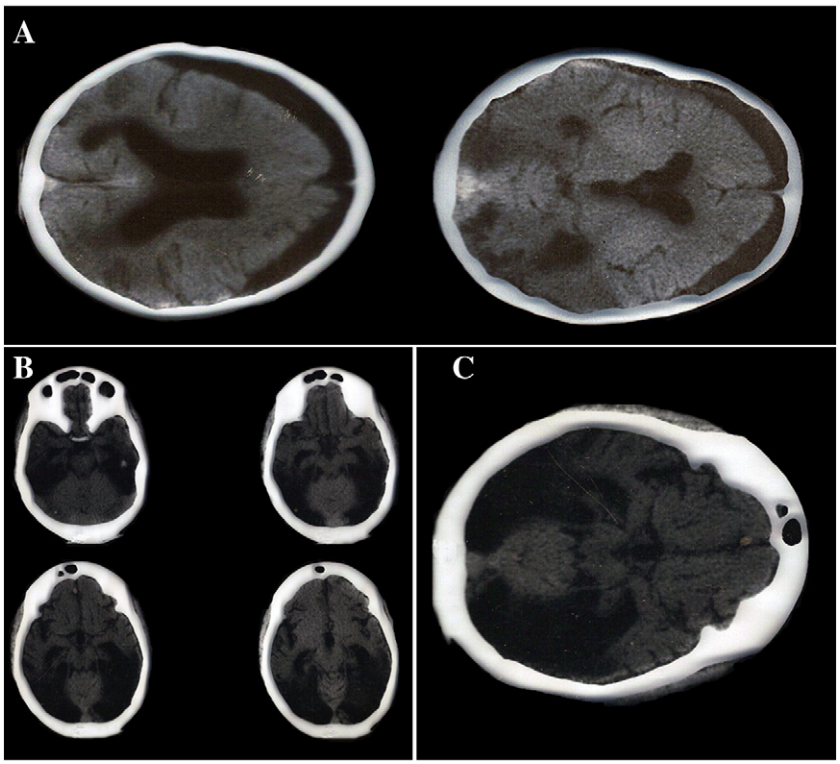

Fig. 6. Case 2. A: images from a CAT scan study performed 3-months after TBI. Cortico-subcortical atrophy, bilateral fronto-parietal hygroma and temporo-occipital porencephaly. B: images from different sections in a new CAT scan study performed 3 years after TBI. Notice the important bilateral lack of parenchyma in all brain lobes. C: magnification of one of the images shown in B. The loss of brain parenchyma is evident, mainly at parieto-temporo-occipital lobes.

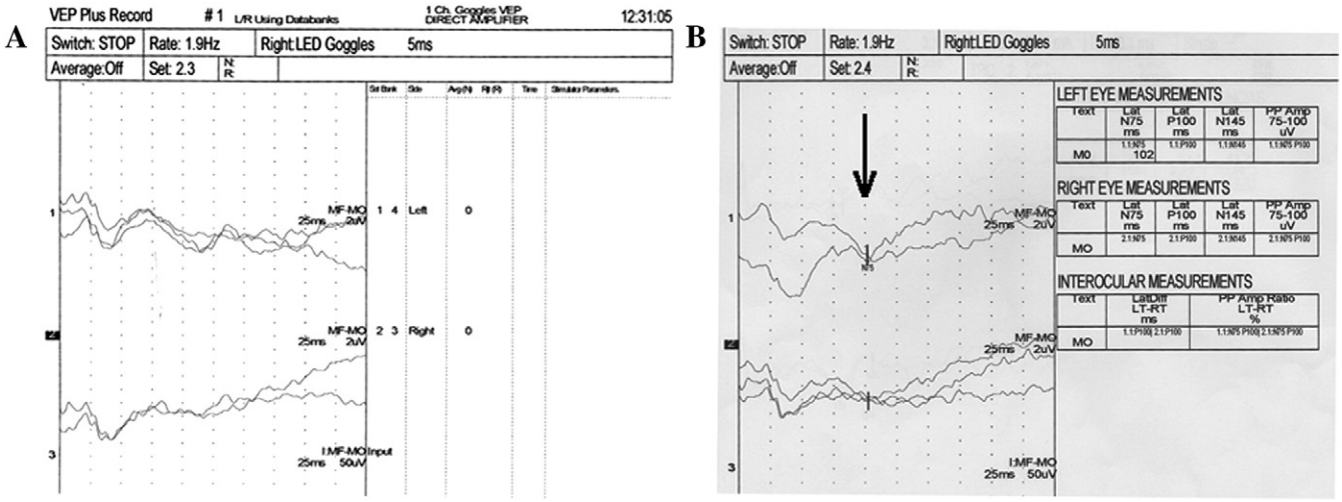

Fig. 7. Case 2. Visual evoked potentials performed with LED goggles before commencing the treatment (A) and 9-months after commencing it (B). A: no peaks detected. B: An N75 peak was detected in the left eye at a latency of 102 ms (black arrow). No peaks were detected in the right eye.

At the end of treatment period (12 months) the patient was able to walk alone with the help of a cane. He also showed clear improvements in his cognitive affectations: memory and attention significantly increased and expressive language clearly ameliorated. Aggressivity decreased and the patient was more confident to interact with the environment. All these findings are showed in Table 1. Plasma IGF-1 values were 201 ng.mL^{-1} before commencing the GH treatment and 296 ng.mL^{-1} after finishing it. No significant side effects were observed throughout the treatment period.

Case 6. Male, 27 years old. High-speed motor vehicle accident at age 16 resulting in severe TBI. MRI study 1 year post-injury revealed diffuse axonal lesion, brain stem affectation, right temporal lobe encephalomalacy and right parieto-occipital gliosis.

At admission, the patient showed right hemiplegia and impaired motor coordination and standing balance, leading to need of the assistance of a person to walk. Cognitive (mainly lack of attention and recent memory) mood alterations and dysarthria were also present. No pituitary hormonal deficits were observed. The patient was scheduled for

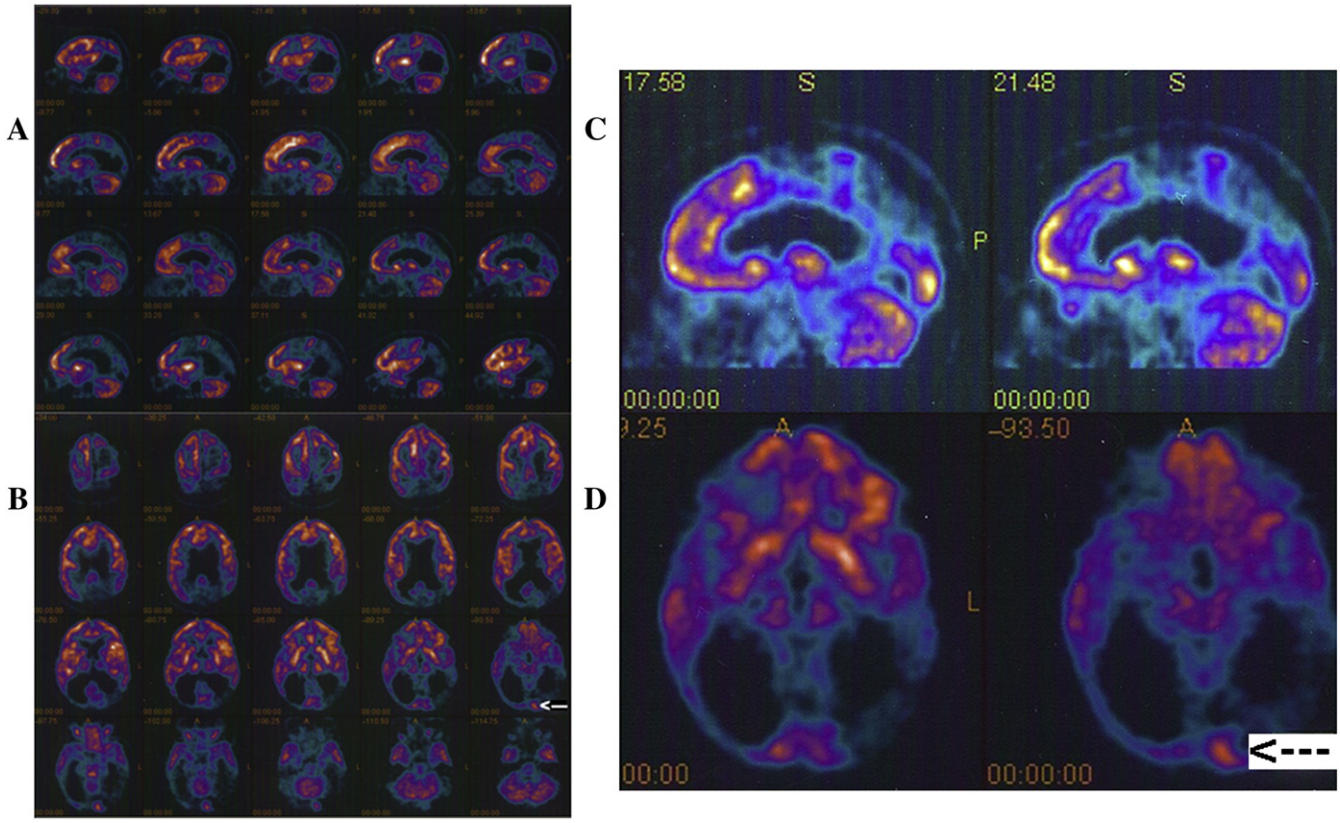

Fig. 8. Case 2. Cerebral PET study performed 12-months after commencing GH treatment and visual stimulation. The patient was given 250 Mbq F18-FDG, iv, and 45 min later she was stimulated with repetitive white light flashes for 15 min (similar to those used for daily visual stimulation). A: sagittal sections. B: transversal sections. C: magnification of two images shown in A. D: magnification of two images shown in B. Black arrow signals metabolic activity in the left occipital lobe at the level of cuneus/calcarine fissure.

GH treatment (maximal dose 1 mg/day), physiotherapy, speech therapy, occupational therapy and psychomotor stimulation.

Ten months after commencing the treatment dysarthria was ameliorated, attention and recent memory improved and the patient was capable of walking without any aid (Table 1). GH treatment did not induce any significant adverse effects, and plasma IGF-1 values were 188 ng.mL^{-1} (pre-treatment) and 213 ng.mL^{-1} (post-treatment).

Case 7. Female, 25 years old. She was hit by a car at age 18 resulting in severe TBI and cardiorespiratory arrest for an unspecified time, leading to a secondary hypoxic–ischemic encephalopathy and a vegetative state. MRI study performed 2 years post TBI showed corticosubcortical atrophy with ventriculomegaly, and hyperintense signals in both centrum semiovale and periventricular white matter.

At admission, the patient remained in vegetative state. Tetraplegia was also present, without cephalic and trunk motor control; together with anarthria, blindness and neurogenic dysphagia requiring tracheostomy for feeding. GCS score was 9. Blood tests demonstrated GHD. The patient was scheduled for GH treatment (maximal GH dose 1 mg/day) and rehabilitation consisting of physical therapy, speech therapy, psychomotor stimulation and visual stimulation.

After 12 months of treatment the patient presented some conscious improvements and was able to follow some simple commands. Cephalic and trunk motor control was also improved and the FOAMS score increased from 1 to 4 (Table 1). Despite these improvements, blindness persisted and the patient was unable to stare at a person or follow the movement of a light. GCS score at this time had increased to 13–14. Plasma IGF-1 values increased from 22 ng.mL^{-1} at the beginning of treatment to 236 mg.mL^{-1} at the end of this 12-month period. No significant side effects were observed. The patient continues with physical and cognitive rehabilitation.

Case 8. Male, 16 years old. Skiing accident at age 15, with severe TBI (GCS 3), remaining in coma for 6 months. CAT scan and MRI studies 1 month after TBI showed punctate hemorrhage in frontal and left brain regions, centrum semiovale and diffuse axonal injury.

At admission the patient presented with spastic tetraplegia and severe mood and cognitive impairments (lack of inhibition, temporo-spatial disorientation and loss of recent memory). Facial hemiparesia, dysarthria, and tongue paralysis with sialorrhea were also present. No pituitary hormonal deficits existed. The patient was scheduled for GH treatment (maximal GH dose 1 mg/day) and rehabilitation consisting of physical therapy, speech therapy, psychomotor stimulation and occupational therapy.

Eight months after the onset of the treatment, spasticity had decreased and balance improved, and the patient was scheduled for surgery because of the shortening of tendons leading to irreducible extension of his left hand and a persistent flexion of his right upper limb (15°) which impeded him to walk alone. A strong cognitive improvement was also assessed (Table 1). Facial mobility and symmetry improved from 3 to 5 according to the classification of House and Brackmann (1985). Dysarthria also improved since tongue paralysis disappeared and sialorrhea decreased from grade 3 to 1 according to the Thomas-Stonell and Greenberg classification for assessment of drooling (Thomas-Stonell and Greenberg, 1988). Therefore, the intelligibility test for facial and speech improvements showed a change from 2 to 4 (Monfort and Juárez, 2001). No side effects were observed during the treatment. Plasma IGF values were 234 ng.mL^{-1} (pre-treatment) and 266 ng.mL^{-1} (post-treatment).

Case 9. Male, 31 years old. High-speed motor vehicle accident at age 29 resulting in severe TBI together with traumatic dissection of the carotid artery and cardiorespiratory arrest for no less than 9 min. No other medical records were provided.

At admission the patient remained in vegetative state. Tetraplegia was also present, without cephalic and trunk motor control; together with anarthria and neurogenic dysphagia which required tracheostomy for feeding. GCS score was 8. Blood tests demonstrated GHD. The patient was scheduled for GH treatment (maximal GH dose was 1 mg/day) and rehabilitation consisting of physical therapy, speech therapy and psychomotor stimulation.

After 12 months of treatment, the patient acquired cephalic and trunk motor control. Conscientiousness also improved and he was also able to follow simple commands and achieved some degree of vocalization. FOAMS score improved from 1 at admission to 5 (Table 1). Plasma IGF-1 values were 62 ng.mL^{-1} at the beginning of treatment and 292 ng.mL^{-1} 12 months later. The only side effect observed during treatment was the detection of gallstones that were removed with surgery. However, this side effect is unlikely to be secondary to GH administration (maximal dose 1 mg/day). This patient continues with physical and cognitive rehabilitation.

Case 10. Female, 24 years old. She was hit by a car at age 21 resulting in severe TBI and remaining in coma for 4 months. MRI study 2 years after TBI showed multiple traces of hemorrhage in both cerebral hemispheres, central midbrain and right cerebellar hemisphere.

At admission in our center, clinical assessments showed a spastic tetraparesis, neurogenic dysphagia, aphonia, and severe cognitive impairments with loss of recent memory and temporo-spatial orientation. The existence of diplopia was also observed. Blood tests detected GHD. The patient was scheduled for GH treatment (maximal GH dose 1 mg/day) and rehabilitation consisting of physical therapy, speech therapy, psychomotor stimulation, occupational therapy and visual stimulation.

Twelve months after commencing the treatment, the patient gained cephalic and trunk motor control being capable of sitting. She also improved from her spastic tetraparesis and began to walk with the assistance of a person, and recovered the ability of manipulating objects with both hands (Table 1). Dysphagia disappeared (FOAMS increased from 2 to 6); and recent memory and temporo-spatial orientation were recovered (Table 1). Diplopia was not corrected. No side effects were observed during GH treatment. Plasma IGF-1 values increased from 29 ng.mL^{-1} (pre-treatment) to 243 ng.mL^{-1} (post-treatment). This patient continues under rehabilitation.

Case 11. Male, 26 years old. High-speed motor vehicle accident at age 24.5 resulting in severe TBI (GSR 3), remaining in coma for 5 months. No other medical records were provided.

At admission in our center clinical assessments showed dystonic tetraplegia, expressive aphasia, severe cognitive impairments and amaurosis. A CAT scan performed at this time showed a left temporal subdural hematoma with middle line deviation and generalized cerebral edema with ventricular collapse. A second CAT scan performed 3-months later revealed extense cystic cavities affecting the left brain hemisphere. No pituitary hormonal deficits were detected. The patient was scheduled for GH treatment (maximal GH dose 1 mg/day) and rehabilitation consisting of physical therapy, psychomotor stimulation, speech therapy, occupational therapy and visual stimulation.

One year after the onset of the treatment clinical assessments (Table 1) showed expressive language improvement; improved cognitive functions (specially memory, orientation and reasoning) and motor improvements (standing balance and walking with the help of another person). Blindness persisted despite improved VEPs (visual evoked potentials). No side effects were observed due to GH treatment. Plasma IGF-1 values were 285 ng.mL^{-1} before commencing GH administration and 319 ng.mL^{-1} after interrupting it. This patient continues under rehabilitation.

Case 12. Male, 26 years old. High-speed motor vehicle accident at age 18 resulting in severe TBI. Medical reports from this time were not provided.

At admission the patient presented left spastic hemiplegia, ataxia, dysarthria, and a slight cognitive impairment. The patient was able to walk with the aid of a cane, but a clear lack of equilibrium existed, so

falls were very frequent and the patient was practically dependent for most of daily life activities. No pituitary hormonal deficits were found in blood sample analysis. The patient was scheduled for GH treatment (maximal GH dose 1 mg/day) and rehabilitation consisting of physical therapy, speech therapy, psychomotor stimulation and occupational therapy.

Six months after commencing the combined treatment the most significant change observed was at motor level. Ataxia clearly decreased and the patient was able to walk alone without any support. Dysarthria also improved and cognitive functions were ameliorated (Table 1). All these led to an independent life, although some motor and speech deficits still exist. Therefore he continues under rehabilitation. No side effects were observed. Plasma IGF-1 levels were 197 ng.mL^{-1} before commencing GH administration and 242 ng.mL^{-1} 6 months later.

Case 13. Female, 6 years old. She was hit by a car at age 4 resulting in severe TBI (GSC 3) and remaining in coma for 4 months. A MRI study performed 1.5 years post-TBI showed a bilateral diffuse axonal injury and important affectations in right lenticular nucleus, left thalamus and corpus callosum body and splenium.

Clinical assessments at admission in our center demonstrated the existence of dystonic tetraplegia, ataxia, dysarthria, anisocoria, sialorrhea, and moderate cognitive impairment. No abnormalities were found in pituitary hormone secretion. The patient was scheduled for GH treatment (maximal GH dose 0.7 mg/day) and rehabilitation consisting of physiotherapy, speech therapy, psychomotor stimulation, occupational therapy and visual stimulation.

Table 2 shows the motor and cognitive improvements reached in this case after 8 months of treatment. Dystonia and ataxia decreased and the patient was able to walk alone (under supervision) for about 200 m. Tool manipulation, reading and expressive language were also improved. Sialorrhea decreased, dysarthria ameliorated and anisocoria disappeared. No side effects were observed during GH treatment and plasma IGF-1 changed from 119 ng.mL^{-1} (pre-treatment) to 251 ng.mL^{-1} (post-treatment). This patient continues under rehabilitation.

Results

Results of this review have been explained for each individual case report. However it is important to remark here that independently of the time elapsed since TBI occurred and of the time the combined treatment of GH and rehabilitation commenced and of the severity of TBI, all patients experienced improvements in his/her motor and/or cognitive affectations.

Age, ethnical and cultural proceedings were very different in the population studied. There were engineers, university students, musicians, workers, or people without secondary studies. Thus, in some cases clinical assessment tests had to be different with those carried out in the majority of cases. Moreover, two patients were in vegetative state and another one showed a very low degree of consciousness, therefore only FOAMS test could be performed on them. In addition, Case 13 was a child, so the tests in her case had to be those specific for children. This is the reason why results from this patient are presented in a separate Table (Table 2).

Table 2
Clinical assessments in the child studied.

Case/time	GHD	GMFM		BDIST		Intelligibility test	
		Pre	Post	Pre	Post	Pre	Post
13/2 y	—	90/264	178/264	27	50	2	4

Notes. Case/time: patient number in case report/time elapsed since TBI and admission (y = years). GMFM: Gross Motor Function Measure. BDIST: Battelle Developmental Inventory Screening Test. Intelligibility test for facial and speech improvements. GHD: GH deficiency. — = no GHD was detected; pre: pre-treatment; post: post-treatment.

Mean time since TBI occurred and treatment commenced was 4.8 years (SD: 3.8).

Mean time of treatment at which clinical assessments were made was 9.7 months (SD: 2.4).

GHD was found in 5 out the 13 patients reviewed. Neurogenic dysphagia existed in 5 patients; interestingly all of them were GHD, most likely because of the nature of TBI they suffered. In all these cases very significant improvements were achieved in swallowing, as Table 1 shows, despite the fact that two of them were in a vegetative state and another one had a very low degree of consciousness.

Cognitive improvements were, in general, better than motor improvements, and they appeared earlier than the latter.

Plasma IGF-1 values (mean ± SD, ng.mL^{-1}) were markedly lower in GHD (40.6 ± 16.9) than in non GHD patients (219.2 ± 60.9), but at the end of the GH treatment period the values were similar between them: GHD 275.6 ± 35.6, non GHD patients 270.2 ± 64. Plasma IGF-1 significantly increased after GH treatment both in GHD ($p<0.01$) and non GHD patients ($p<0.05$), however percentage increase was significantly higher ($p<0.01$) in GHD (mean: 654%) than in non GHD (mean: 28.8%) patients. Hematocrit slightly increased in all the patients after GH treatment.

The best results were obtained in patient 1, since he achieved full functionality at the end of the treatment period, perhaps because of the nature of his TBI and/or his age; in addition, treatment in him commenced very soon after TBI, unlike what happened in the other patients.

Discussion

From our results it seems clear that GH treatment significantly contributed to the improvements achieved by physical and cognitive rehabilitation in the patients studied. While it is difficult to exactly delimitate the role played by the hormone, all the patients in this report (except for Case 1) had been treated for years with similar rehabilitation procedures, but without significant improvement. However all patients experienced improvements in their motor and/or cognitive affectations in response to the combined rehabilitation and GH treatment, independently from the time elapsed since TBI, or the severity of the TBI. Therefore, and since GH treatment was the only significant variable introduced in the results described here, it is reasonable to assume that GH is providing the means for the adequate response to the demands requested by the motor and sensory stimulation.

It is presently clear that GH is a pleiotropic hormone expressed not only in the pituitary but in many other tissues, including the CNS (Devesa et al., 2010). Thus, far beyond of its classical actions on longitudinal body growth and intermediate metabolism, GH may act as a local factor that plays an important role in the regulation of cell proliferation and survival (Costoya et al., 1999; Sanders et al., 2009; McLenachan et al., 2009; Aberg et al., 2009). The hypothesis that GH may play a role on brain repair was postulated several years ago, and a number of preclinical and clinical studies demonstrate the positive effects of GH treatment on adult neurogenesis in both laboratory animals and GHD patients (Scheepens et al., 1999, 2001). In addition, the expression of both GH and GH receptor (GHR) is strongly upregulated after brain injury and specifically associated with stressed neurons and glia (Scheepens et al., 2000; Christophidis et al., 2009; Li et al., 2011). However, the physiological role of GH at the central level and, in particular, its possible contribution to the reparation of neurologic injuries remains poorly understood.

Accumulating evidence suggests that the beneficial effects of GH on neural repair may be exerted through different mechanisms that include the regulation of the proliferation, survival, differentiation and migration of both neural progenitors and newly-formed neurons. The ability of GH to promote the proliferation of neural precursor is supported by different in vitro and in vivo findings. GH treatment

promotes proliferation of both human fetal (Pathipati et al., 2011) and adult mice neural stem cells (NSCs) (McLenachan et al., 2009; Devesa et al., 2011b). Peripheral administration of GH has been also demonstrated to induce cell proliferation in the brain of both adult hypophysectomized (Aberg et al., 2009) and normal rats (David Aberg et al., 2010); and to increase the proliferative response of hippocampal progenitors to kainate-induced injury (Devesa et al., 2011a).

However, besides the importance of this neuroproliferative response of GH-induced neural repair, the results obtained with some cognitive deficits suggest that the proliferation of neural precursors is not the only repairing mechanism triggered by GH treatment. Cognitive improvements were, in general, better and appeared earlier than motor improvements, thus reinforcing the importance of GH in the recovery of this kind of disabilities. A role for GH in these patients is also supported by the fact that GH deficiency (GHD) is frequently associated with reduced cognitive performance that may be reversed by GH replacement (Maruff and Falleti, 2005; van Dam, 2006; Falleti et al., 2006; Nieves-Martinez et al., 2010). A positive effect of GH treatment on cognitive functions has been also reported in TBI GHD patients (High et al., 2010; Reimunde et al., 2011), a finding also supported by our data in this study. Although we do not presently know the mechanisms underlying this effect, an intriguing possibility is that GH treatment is increasing the number of newly formed neurons in the hippocampal dentate gyrus, a zone related with recent memory. This mechanism would also explain the positive effect of GH treatment in TBI patients without GHD given the ability of the exogenous GH to cooperate with the endogenously produced hormone to increase the proliferation of hippocampal neural precursors (Aberg et al., 2009, Devesa et al., 2011a). However, the improvement observed in other cognitive processes such as attention or concentration cannot be attributed to newly formed neurons, because they appear too early after the onset of the treatment, both in this study and in previous studies from our group (Reimunde et al., 2011; Devesa et al., 2011a, 2011b). Pituitary GH release is mainly dependent on the negative control by hypothalamic somatostatin. In turn, somatostatin release is negatively controlled by alpha$_2$-adrenergic pathways (Devesa et al., 1992). When GH is released it stimulates somatostatin secretion and this leads to an inhibition of a subsequent GH secretion, so that GH release from the pituitary occurs episodically. Therefore, exogenous GH administration would lead to enhanced somatostatin secretion and this, in turn, would produce increased noradrenaline (NA) synthesis and release (Devesa et al., 1992). Since dopamine (DA) is the precursor of NA synthesis, any increase in plasma GH would lead to enhanced DA and NA turnover. That is, according to these concepts, GH administration would affect some neurotransmitter pathways and this might explain the early responses observed after commencing a treatment with GH. These concepts are shown in Fig. 9.

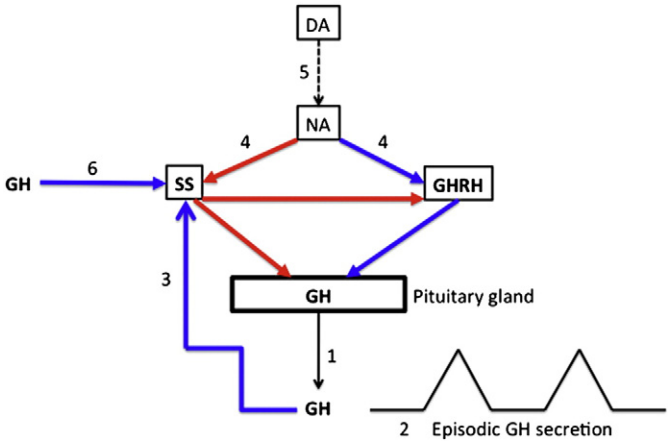

Fig. 9. Neuroregulation of GH secretion. GH is negatively controlled by hypothalamic somatostatin (SS) and stimulated by hypothalamic GHRH, which in turn is negatively controlled by SS too. Hypothalamic release of SS and GHRH is rhythmic and alternant, so that SS withdrawal allows GHRH supply to the pituitary gland leading to GH release (1). Therefore, GH secretion occurs episodically (2). Increased secretion of GH stimulates SS secretion (3). This leads to increased NA synthesis (4) for blocking SS release, acting on alpha2-adrenergic receptors, and stimulating GHRH secretion (4). NA synthesis is produced from DA (5), thus increased NA needs leads to increased DA synthesis and metabolism. The administration of exogenous GH (6) will reproduce the physiological effects of GH on SS secretion and thus on DA and NA metabolic turnover. Blue arrows = stimulation. Red arrows = inhibition.

Therefore, other GH-induced mechanisms may cooperate in the production of new neurons and synapsis to promote the cognitive recovery in TBI patients. Despite the prominent effects of GH on the proliferation of neural precursors, it has been suggested that it may actually play a more important role in the regulation of survival, differentiation, or even migration of newly formed neurons (Christophidis et al., 2009). Consistent with the role of GH in the regulation of cell survival, GH prevents the apoptotic death of both mature neurons (Möderscheim et al., 2007; Byts et al., 2008; Silva et al., 2003) and primary neurospheres derived from embryonic mouse NSCs (van Marle et al., 2005). On the other hand, treatment of NSCs with a GHR antagonist, significantly increases apoptotic death (Devesa et al., 2011c). Finally, the survival of newborn neurons in the subgranular zone of adult rat dentate gyrus is impaired as a result of GH deficiency (Lichtenwalner et al., 2006), while elevated GH levels within the hippocampus reduce apoptosis (Sun and Bartke, 2007). All these neuroprotective effects would help to understand some of the short-time effects of GH treatment.

A role for GH in the regulation of neuronal differentiation is supported by several reports indicating that human GH enhances the differentiation of neural precursors (Ajo et al., 2003; Lyuh et al., 2007) and stimulates neurite initiation and arborization in embryonic hippocampal neurons by activating the PI3K/Akt signaling pathway (Byts et al., 2008). However, GH has been recently reported to not significantly affect neurite regeneration in differentiated postmitotic neurons derived from fetal human spinal cord neural precursors (Koechling et al., 2011). Remarkably, GH concentrations that accomplish to promote differentiation of neural precursors have been reported to reduce neuronal proliferation (Ajo et al., 2003; Lyuh et al., 2007), thus suggesting that, depending on the dose, GH may exert a dual effect on the proliferation and differentiation of neural precursors. In this regard, it is interesting to indicate that neurosphere cultures derived from GHR knockout mice proliferate less than those from wild type mice, yet they exhibit accelerated neuronal differentiation (McLenachan et al., 2009). Conversely, treatment of neurospheres derived from newborn or adult mouse neural stem cells with a GH that stimulates cell proliferation (Christophidis et al., 2009), significantly reduces neuronal differentiation (Turnley et al., 2002; Scott et al., 2006).

Finally, a possible effect of GH on neural stem cells migration is less clearly established. It has been reported that GH delivered icv to rats subjected to ischemia localizes to migratory neuroblasts (Pathipati et al., 2009). Significant post-injury up-regulation of GHR on migrating neuroblasts (Christophidis et al., 2009) and in neurons moving from neurospheres obtained from the dentate gyrus of 9-day old mice has also been reported (Devesa et al., 2011b). Of interest, a recent study (Pathipati et al., 2011) proves a direct role for GH in regulating NSC migration, suggesting that GH-induced NSC migration is dependent on the activation of prolactin receptor by GH.

Despite the ability to directly stimulate signaling pathways that may lead to the promotion of neurogenesis or neurorepair, we cannot rule out the possibility that GH actions may be exerted, at least in part, through indirect mechanisms. These include the synthesis and/or release of a number of factors with known neurotrophic activity, such as IGF-1, epidermal growth factor (EGF) or erythropoietin (EPO); or changes in neurotransmitter turnover, as indicated before.

IGF-1 has been recognized for years as one of the major mediators of some of the GH actions, mostly involved in the control of

body growth and tissue remodeling. However, other aspects of the IGF-1 biology are gradually emerging and suggest that this growth factor has a prominent role in brain function (Torres-Aleman, 2010). IGF-1 is essential for brain development (Russo et al., 2005; Popken et al., 2005). IGF-1 stimulates proliferation of neural progenitors, survival of neurons and oligodendrocytes, and differentiation of neurons (including neuritic outgrowth and synaptogenesis) and of oligodendrocytes (including expression of myelin gene proteins and myelination). Consequently, brain growth is increased with IGF-1 overexpression and reduced as a result of decreased IGF-1 signaling (for a detailed review about IGF-1 effects at the central level, see: D'Ercole and Ye, 2008). A phase II safety and efficacy trial indicated that intravenous therapy with continuous IGF-1 improved clinical outcome in patients with moderate-to-severe head injury (Hatton et al., 1997). Children with IGF-1 deficiency due to GH insensitivity (Laron syndrome) show central nervous system underdevelopment (Laron, 2001; D'Ercole and Ye, 2008); treating these children with IGF-1 increases head growth and improves brain functions (Laron and Klinger, 1994). These and other data (Aberg et al., 2000; Anderson et al., 2002) suggest that the adult brain has an external input from serum IGF-1, where this anabolic peptide is abundant. However, it is uncertain that the increase observed in circulating IGF-1 levels after GH treatment in some of our patients is enough to be responsible for the responses observed. Early reports have also described IGF-1 and IGF-1R expression in neural progenitor cells (Drago et al., 1991; Garcia-Segura et al., 1991; D'Ercole et al., 1996). Therefore, the possibility also exists that local production of IGF-1, which has been recognized to exert pleiotropic effects on adult brain function (Torres-Aleman, 2010) may be responsible for some of the beneficial effects observed. In this regard, it is interesting to indicate that, IGF-1 has been also proven to be an important modulator of brain activity, including higher functions such as cognition, and to regulate genes involved in microvascular structure and function and synaptic plasticity (Torres-Aleman, 2010, Yan et al., 2011). However, there are also evidences supporting that GH has IGF-1-independent effects (Pathipati et al., 2011). In fact, studies in rats submitted to moderate and severe hypoxia showed that the spatial distribution of the neuroprotection conveyed by GH correlates with the spatial distribution of the constitutive neural GHR, but not with the neuroprotection offered by IGF-1 treatment in this model. Therefore, some of the neuroprotective effects of GH are mediated directly through GHR and do not involve IGF-1 induction (Scheepens et al., 2001).

Epidermal growth factor (EGF) has been proven to be an effective mitogen for inducing neurogenesis in animal models, both under basal conditions or after experimental injuries (Calza et al., 2003; Türeyen et al., 2005). Recent findings have shown that cells derived from subventricular zone (SVZ) actively respond to EGF stimulation becoming highly migratory and proliferative contributing to myelin repair (Gonzalez-Perez and Alvarez-Buylla, 2011). Since GH induces both EGF and EGFR expression and EGFR activation in many territories (Yamauchi et al., 1998; Pan et al., 2011), it is tempting to speculate the some of the GH effects may be attributed to this fact. However, it is noteworthy to indicate that EGF-induced neurogenesis is not sustained, and any positive effect of EGF must be attributed to a neuroprotective rather than neurogenic effect (Sun et al., 2010).

The hematopoietic growth factor erythropoietin (EPO) is another factor involved in neurogenesis. Both EPO and its receptor (EPOR) are detected in a number of brain areas during brain development as well as in vitro in neurons, astrocytes, oligodendrocytes, microglia and cerebral endothelial cells, and both are upregulated after hypoxia or ischemia. Other stimuli such as hypoglycemia or IGF-1 also lead to increased expression of EPO (Byts and Sirén, 2009). Peripherally administered EPO crosses the blood–brain barrier and in the brain activates anti-apoptotic, anti-oxidant and anti-inflammatory signals in neurons, glial and cerebrovascular endothelial cells, and stimulates angiogenesis and neurogenesis. Owing to these effects, EPO exerts potent tissue-protecting actions in experimental models of stroke, cerebral hemorrhage, traumatic brain injury, and neuroinflammatory and neurodegenerative diseases (Byts and Sirén, 2009). In addition, specific blockade of EPOR in the brain leads to deficits in neural cell proliferation and neuronal survival during embryonic development and in post-stroke neurogenesis in the adult brain (Chen et al., 2007; Tsai et al., 2006). Finally, both in vivo and in vitro findings indicate that EPO amplifies stroke-induced oligodendrogenesis that could facilitate axonal remyelination leading to functional recovery after stroke (Zhang et al., 2010). Since GH induces EPO release from kidneys (Sohmiya and Kato, 2001), EPO may be mediating, at least in part, some of the effects observed.

Though the understanding of the mechanisms responsible for the beneficial effects of the combined GH and rehabilitation therapy is a very important issue, it is also of interest to focus on where these actions are exerted. That is, regardless of the necessary central neurological improvements, some of the clinical results obtained have to be explained on the basis of a peripheral neurological driving improvement. This is best exemplified by the clinical improvement observed in TBI patients with neurogenic dysphagia.

Neurogenic vocal cord paralysis is usually caused by injury of the vagus nerve (X) or one of its branches, and frequently results in manifest clinical disabilities such as aspiration, dysphagia and dysphonia (Saito et al., 2003; Mori et al., 2007). Up to now, there is no effective treatment for vocal cord paralysis, and reinnervation procedures have limited effects on functional recovery (Shiotani et al., 1999; Isshiki, 2000; Saito et al., 2003; Mori et al., 2007). In a previous report (Devesa et al., 2009) we described an important improvement of swallowing performance (together with recovery from other neurological sequelae secondary to oncological brain surgery) in an adult GHD patient after intensive physical therapy, speech therapy and GH treatment. Since the patient had undergone intensive physical rehabilitation for a 15-year period with no significant improvement, we concluded that GH replacement therapy was responsible for her improved response to rehabilitation. Results presented herein also support this hypothesis, and lead to the possibility that some of the GH effects are exerted at the periphery. In this regard, we also recently demonstrated (Devesa et al., 2012) that GH treatment is able to induce functional regeneration of the sciatic nerve after surgical cut and repair in rats. It has been recently shown that GH can act, in an autocrine and/or paracrine manner, as a signaling molecule to promote axonal growth during the development of the nervous system (Baudet et al., 2009). Even assuming that reinnervation occurred, as clinical data demonstrated, it is unclear how misdirection of the nerve fibers was corrected by GH treatment. As suggested before, the possibility exists that speech therapy generated a demand and GH (alone or in cooperation with other neurotrophic factors, as indicated in the Introduction) provided the means for obtaining a response.

It has been reported that spontaneous reinnervation may be possible, since some studies using animal models indicate that there is a strong tendency for reinnervation after recurrent laryngeal nerve injury. However, the existence of spontaneous reinnervation can be completely ruled out in our study because of several reasons. First, while spontaneous recovery may occur after recurrent laryngeal nerve injury, it is unlikely to occur when more proximal vagus nerve involvement exists (Hydman et al., 2007; Woodson, 2007). In addition, spontaneous innervations have been described in peripheral nerve injuries, but not after CNS lesions, as what occurs in the patients in this study. Finally, but more importantly, the long period of time from the injury until the onset of the treatment in 4 of the 5 patients clearly excludes spontaneous reinnervation as a factor responsible for the improvements observed.

VEP recorded in patient number 2 indicates that GH treatment and visual stimulation led to a significant appearance of sensorial

neurological transmission from the retina to the visual cortex. In addition, brain PET study indicates that a certain degree of neurological input is reaching the V1 zone from the retina and activating some cellular groups there. Furthermore, a detailed analysis of PET study indicates the existence of certain metabolic activity in cortical areas receiving information from the magnocellular visual pathway coming from the retina (V1, V2, V3 and V5). These perceive global aspects of a stimulus and its movements, a situation compatible with the clinical improvements observed in this patient 11 years after her absolute blindness. How to explain that? On the one hand, since the patient had a bilateral optic nerve atrophy, a certain degree of nerve recovery had to be achieved. Together with it, the visual occipital cortex, previously shown to be practically destroyed after the injury, had to be partially recovered too, as metabolic activity in PET study shows.

As described before, IGF-1, EPO and EGF, might account for an acute recovery of visual pathways damaged after a TBI, but we cannot know whether any or all of them might have participated in the results obtained in Case 2 after GH treatment and visual stimulation commenced 11 years after her TBI. The increase observed in circulating IGF-1 levels after GH treatment in this patient does not seem to be important enough to be responsible for the responses obtained. Moreover, this patient was overweight both before commencing GH treatment and after it, therefore malnutrition cannot be responsible for absent VEP waves.

A report describes modest increases in VEP grating acuity in children with cerebral visual impairment and a history of neonatal hypoxic–ischemic encephalopathy (Lim et al., 2005), but these cannot account for VEP results in Case 2. Both the nature of her brain injury and the time between it and the start of the treatment discard any spontaneous recovery.

From our data, it is also important to establish the relevance of the existence of a possible GHD in TBI patients in the outcome of the clinical treatment. The existence of acquired GHD after TBI is a quite common finding, and a recent study (Moreau et al., 2011) describes that GHD was more prevalent than other pituitary deficiencies in a cohort of 55 patients studied at least 1 year after TBI. In contrast, other studies (Kokshoorn et al., 2011) reflect that only 3 of 112 patients with TBI, which occurred at least 1 year before, had severe GHD. Most likely the complexity of GH neuroregulation (Devesa et al., 1992) is responsible for these differences. The existence of a GHD after TBI may constitute an important argument to support the use of GH treatment in these patients, as we previously demonstrated in GHD patients with neurological disabilities (Devesa et al., 2009, 2011a; Reimunde et al., 2010, 2011). However, in the present report, we found GHD in only 5 out of 13 patients, and similar positive results were obtained in both types of patients. Therefore, it is unlikely that the beneficial effects of GH administration are secondary to the replacement of a GHD and, in fact, we (Devesa et al., 2011b) and others (Aberg et al., 2009) have demonstrated that GH treatment may cooperate with locally-produced GH in promoting neural repair in rats without GHD.

Conclusions

In summary, our data indicate that GH administration is useful when combined with the adequate rehabilitation in TBI patients, independently that GHD exists as a consequence of brain injury. These data in human patients do not allow us to establish how and where GH is acting on brain repair, or if its effects are produced by itself or by cooperating with a number of neurotrophic factors induced by the hormone, particularly IGF-1.

In our study, plasma IGF-1 significantly increased after GH treatment, therefore the possibility exists that these results could be produced by this insulin-like growth factor. However, percentage increase of plasma IGF-1 was markedly higher in GHD than in non GHD TBI patients, while absolute values of plasma IGF-1 and clinical results obtained were similar in both group of patients; thus it is unlikely that plasma IGF-1 increase may be the unique factor responsible for the improvements observed. We cannot discard the idea that GH induced central IGF-1 expression, a possibility that is difficult to be tested in human patients.

With regard to the mechanisms triggered by GH and rehabilitation, most likely they involve neurogenesis, brain plasticity and changes in some neurotransmitter metabolism. In the case of increased neurogenesis, it is well known that neuronal precursors may release trophic factors capable of activating silent neurons in penumbra areas and establishing new functional networks. Tractography studies will clarify whether GH facilitates the development of these new networks.

In any case, it seems to be clear that GH treatment has much more advantages than any other treatment demonstrated until now. Although the positive effects of the administration of the hormone together with physical and sensorial stimulation can be seen when the treatment commences years after TBI, it is likely that a prompt administration of GH after brain injury will improve the results achieved. However, it is feasible that GH treatment will not be a solution in many cases and not all the disabilities will be resolved. GH administration for TBI treatment has to be combined with the adequate rehabilitation. GH treatment in this case is safe and short in time, so it is unlikely that any undesirable effects will appear during or after GH administration.

Declaration of interest

The authors report no conflicts of interest in this study.

Acknowledgments

Studies in human patients have been carried out in the Medical Center “Proyecto Foltra” and supported by Fundación Foltra. We acknowledge the work carried out by therapists from this center. The VEP in Case 2 was performed by Professor Jesús Otero (Service of Neurophysiology, University Hospital of Santiago de Compostela (CHUS)), and we sincerely acknowledge his collaboration. The PET study in Case 2 was performed in the Service of Radiology of the University Hospital of Santiago de Compostela. We acknowledge the kind collaboration of Professor Alvaro Ruibal (Service of Radiology) and Dr. J.L. Relova (Service of Neurophysiology) for carrying out this PET study. We acknowledge the financial help of Fundación Juan Entrecanales Azcárate and Fundación Repsol.

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References for the clinical assessments methods shown in Tables 1 and 2

BDIST
FOAMS
GMFM
Intelligibility test
MMSE
WAIS