RESEARCH PAPER

Acta Neurobiol Exp 2018, 78: 352–357
DOI: 10.21307/ane-2018-034

ACTA NEUROBIOLOGIÆ EXPERIMENTALIS

Neurological improvement in patients with chronic spinal cord injury treated with leuprolide acetate, an agonist of GnRH

José Luis Quintanar[1]*, Carmen Díaz-Galindo[1], Denisse Calderón-Vallejo[2], Irma Hernández-Jasso[1], Froylán Rojas[1], Daniel Medina-Aguiñaga[1] and Carlos Olvera-Sandoval[1]

[1] Laboratory of Neurophysiology, Department of Physiology and Pharmacology, Universidad Autónoma de Aguascalientes, Aguascalientes, México,
[2] Department of Morphology, Universidad Autónoma de Aguascalientes, Aguascalientes, México,
** Email: jlquinta@correo.uaa.mx*

It has been reported that gonadotropin-releasing hormone (GnRH), and its analogue leuprolide acetate (LA), have neurotrophic properties; particularly in the regeneration of injured spinal cord in animal models and in the case of a patient with spinal cord injury (SCI). The aim of this study was to establish whether treatment with LA improves sensitivity, motor activity and independence in patients with chronic SCI. Patients were treated LA once a month for six months. They were evaluated at the beginning and at the end of treatment; using a sensitivity and motor impairment scale, according to the American Spinal Injury Association (ASIA), and grade of independence scale; employing the spinal cord independence measure (SCIM). Statistical analysis showed a significant improvement in the ASIA sensory score and the SCIM score when comparing the initial versus final evaluation after six months of LA administration. Some patients showed an increase in frequency of bowel movements. Treatment with LA induces improvements in sensitivity, motor activity and independence in patients with chronic SCI. One advantage of this protocol is that it is a non-invasive method of easy and safe application, with few side effects.

Key words: neuroregeneration, chronic SCI, leuprolide acetate, motor recovery

INTRODUCTION

Spinal cord injury (SCI) temporarily or permanently disrupts neuroanatomical circuitry and can result in severe deficits such as loss of motor, sensory, and autonomic functions. Management of the SCI has change in recent years, both in acute and chronic lesions. There are different small trials performed for the acute and chronic SCI in course, among which are the use of neurotrophic factors, Nogo neutralizing antibodies, N-methyl-D-aspartate receptor modulators and autologous mesenchymal stem cells (Varma et al. 2013).

In animal models of SCI, neurotrophic factors play an important role for improvement of injury nervous system and behavioral effects. In all these models, an acute and intra-lesion administration of the neurotrophic factors is required to show the outcome (Harvey et al. 2015). In recent years, it has been reported that gonadotropin-releasing hormone (GnRH) and its analogue, leuprolide acetate (LA), have neurotrophic properties. *In vitro*, GnRH incubation induced changes in outgrowth, number and length of neurites in rat cerebral cortical neurons and spinal cord neurons (Quintanar and Salinas 2008, Quintanar et al. 2016a). *In vivo*, administration of GnRH improves locomotor activity and bladder function, and increases the expression of neurofilaments in spinal cord in rats with SCI (Calderón-Vallejo et al. 2015, Calderón-Vallejo and Quintanar 2012). Likewise, the administration of LA im-

Received 17 May 2018, accepted 9 December 2018

proves locomotor activity, gait, micturition reflex and spinal cord morphology in rats with SCI (Díaz-Galindo et al. 2015). The use of LA has certain advantages over the natural hormone GnRH. The agonist is less susceptible to proteolysis than GnRH, and has a greater binding affinity to receptors than the natural hormone, increasing its biological activity (Periti et al. 2002). It is also able to cross the blood-spinal cord barrier (Barrera et al. 1991).

Regarding the current clinical therapies and animal trials mentioned above, most of these treatments have an acute approach, which means that the treatment begins immediately after the injury. For clinical purposes, the term "chronic" is used to refer the period after the initial hospitalization (Ditunno and Formal 1994). The chronic SCI has a several systemic complications; these could be a cardiovascular response (orthostatic hypotension, bradycardia), autonomic dysreflexia (cardiac, bladder, bowel, temperature, and sexual dysfunction), pulmonary, hyponatremia, deep vein thrombosis, pressure ulcers, spasticity, neuropathic pain, among others (Sweis and Biller 2017), and most of these complications only have support management.

Currently, and parallel to these previous information, LA is used in humans for diverse clinical applications related to endocrine pathologies; including palliative treatment of prostate cancer, management of endometriosis, as a concomitant therapy for uterine leiomyomata, and central precocious puberty (Wilson et al. 2007). It is well established that the side effects of chronic treatment with LA is a decrease in levels of sex hormones (Mathias et al. 1998). In a preliminary study, independent of the therapeutic effects related to endocrine diseases, we tested the treatment in a patient with chronic SCI and after 12 months of application, sensitivity and motor functions were partially recovered (Quintanar et al. 2016b).

Considering that LA acts as a drug with neurotrophic proprieties, already used in humans for other pathological conditions, able to cross the blood-spinal cord barrier after an intramuscular administration, and with few and well described side effects, the aim of this work was to establish whether treatment with LA improves sensitivity, motor activity and independence in patients with chronic SCI.

METHODS

Study design and site

This is a prospective, non-masked, pilot type phase II clinical trial. The study was carried out at the Clinic of Integral Rehabilitation, Universidad Autónoma de Aguascalientes, México.

Inclusion and exclusion criteria

Inclusion criteria were: aged between 18 to 60 years, diagnosis of chronic SCI (patient who had SCI longer than 5 months) confirmed by clinical evaluation with American Spinal Injury Association (ASIA) impairment scale A or B, computed tomography or magnetic resonance imaging scan of the lesion, and establishment of damage with no evidence of neurological improvement prior the treatment. Patients were excluded from the study if they had any of the following: non-traumatic cause of the lesion (vascular, tumors, surgery, and special cases), impairment health status or terminal disease, and currently participating in another clinical research protocol.

Study protocol

Fourteen patients were included in this study chosen according to inclusion criteria described above. All the participants gave their informed consent in writing and the procedure was carried out in agreement with the local Bioethics Committee of the Universidad Autónoma de Aguascalientes. The patients have a clinical evaluation which include: clinical history, ASIA impairment scale (Ditunno et al. 1994), and grade of independence using the spinal cord independence measure (SCIM) (Catz et al. 1997). Following the initial evaluation, intramuscular administration of 3.75 mg of LA (Lucrin Depot 3.75 mg, Abbott Laboratories, Chicago, IL) commenced. The drug was applied monthly for six months. At the end of six month of treatment, another evaluation was performed including ASIA impairment scale, grade of independence with SCIM and any other finding observation. During the treatment, patients continued with their usual activities, which included physical rehabilitation (all patients for at least 3 hours/week).

Statistical method

The goal of the study was to test the null hypothesis that the mean difference between basal and follow-up (at 6-month treatment) of ASIA sensory, ASIA motor and SCIM is significantly different. The paired t-test (normally distributed data) was used to analyze the difference between basal and follow-up values. For all tests $p<0.05$ was considered significant. Statis-

tical analysis was performed using GraphPad Prism 7.0 for Windows (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

During treatment, none of the patients presented complications related to LA treatment and no severe adverse events were observed. The main characteristics of patients are shown in Table I. The mean age of the patients was 33.4 years and with an average of 31.5 months with SCI, which is considered a chronic pathology. The highest percentage of patients recorded was men and by vehicular accidents (both 85%). The most frequent lesions were at the cervical level (50%) followed by the thoracic (42.8%) and the least frequent were the lumbar (7.1%). Results of ASIA sensory, ASIA motor and SCIM scores before and after of 6 months of treatment are described in Table II. In the sensory analysis of ASIA, 71% of patients reported an increase in the number of dermatomes (at least in one) that responded to the sensory stimulus after the treatment. While in motor ASIA, percentage of patients who increased the degree of mobility and/or strength in at least one muscle group of some limb was 57%. Further, SCIM scores revealed that 92% of the patients improved their level of independence after the treatment. Thus, statistical analysis showed a significant improvement in results of ASIA sensory score (87.2 ± 11 *vs.* 96.7 ± 10.7), ASIA motor score (35.9 ± 5.3 *vs.* 39.4 ± 4.9), and SCIM score (37.5 ± 4.9 *vs.* 41.8 ± 5.0) comparing the initial evaluation and the end (6 months) of LA administration (Fig. 1). Analyzing the recovery percentage of patients with respect to the ASIA maximum score, it was found that the sensory recovery was 4.3% (considering a score of 224 as 100%). The motor recovery was 3.5% and the level of independence 6.3% (considering a maximum score of 100 as 100% for both scores).

In addition to the recovery shown with the ASIA scores, five patients (35%) reported an increase in the frequency of bowel movements related to a greater number of evacuations or a lower use of laxative substances. Also, a patient with a C8 lesion reported a greater sensitivity to changes in temperature (hot and cold) and a lower muscular effort to cough.

DISCUSSION

The effect of the neurotrophin family; nerve growth factor, brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4/5, on neuroregeneration in axons of injured spinal cord is well established (Kadoya et al. 2009). Likewise, it has been reported that the treatment with acidic fibroblast growth factor

Table I. Clinical characteristics of subjects in the study.

Subject	Gender	Age (years)	Mechanism of injury	Level of injury	ASIA score	Injury time (months)
1	M	64	Hernia	L2	B	44
2	F	61	Vehicular	T9	B	15
3	M	33	Vehicular	C7	B	43
4	M	23	Vehicular	C5	A	25
5	M	21	Vehicular	T8	A	43
6	M	39	Vehicular	C6	A	19
7	M	40	Vehicular	C5	A	28
8	M	18	Vehicular	T9	A	28
9	M	25	Vehicular	C4	A	59
10	M	26	Fall	C6	A	21
11	M	29	Vehicular	T8	A	22
12	F	29	Vehicular	C8	A	59
13	M	34	Vehicular	T3	A	6
14	M	26	Vehicular	T7	B	30

ASIA – American Spinal Injury Association. M – male, F – female.

(aFGF) induced significant improvements in ASIA motor and sensory scale scores and functional independence measures at 24 months after procedure (surgery is required for the administration of aFGF) (Wu et al. 2008). Other clinical trials that use thyrotropin-releasing hormone (TRH) with neurotrophic properties, report that an intravenous administration dose of TRH 12 hours after SCI improved significantly the sensor and motor functions evaluated 4 months after the treatment (Pitts et al. 1995).

In the present work, we report that the use of LA to enhance recovery of the human central nervous system is a novel and friendly method that provides a non-invasive pharmacological treatment to patients with chronic SCI. With LA treatment, the degree of recovery is partial; however, for the daily life of patients it is very significant. Of the fourteen patients included in this study, ten showed improvements in their sensory record (71%); eight in the motor function (57%) and thirteen in their SCIM score (92%).

To explain these results, different mechanisms may be suggested by which LA may improve neurological functions. It is possible that the effects obtained by LA administration are due to the activation of receptors for GnRH present in the spinal cord. Previously, the presence of GnRH receptors in spinal cord neurons in animal models such as sheep (Dolan et al. 2003) and rat (Quintanar et al. 2007) has been described, and these receptors respond to stimulation with GnRH (Quintanar et al. 2016a). Once the receptors are activated, biochemical, morphological and functional changes could be generated in the injured nervous tissue.

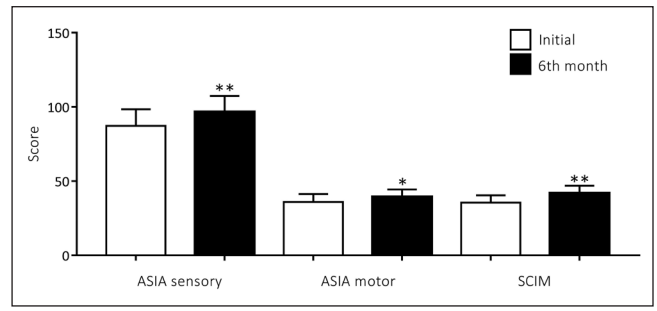

Fig. 1. ASIA scores (sensory and motor) and SCIM score analysis of SCI patients with LA treatment every month for 6 months. Data represent the average ± SEM. The Wilcoxon signed rank test for independent samples was used. ASIA – American Spinal Injury Association; SCIM – spinal cord independence measure. *P< 0.007; **P< 0.001. A paired Student t-test was used to analyses the difference between basal and follow-up values.

Table II. Results of clinical evaluation in the initial and sixth month after LA treatment.

Patient	Sensorial ASIA			Motor ASIA			SCIM			Observations
	Time in months									
	Initial	6th	% Recovery	Initial	6th	% Recovery	Initial	6th	% Recovery	
1	176	192	7.1	64	70	6	71	76	5	
2	108	116	3.6	50	50	0	35	39	4	
3	93	104	4.9	50	54	4	28	35	7	↑ Bowel management
4	40	40	0	1	1	0	0	0	0	Improve cought
5	116	115	- 0.4	50	52	2	52	55	3	
6	64	76	5.4	25	30	5	30	50	20	↑ Bowel management
7	32	49	7.6	9	16	7	11	16	5	↑ Bowel management
8	125	128	1.3	50	50	0	33	47	14	
9	61	80	8.5	10	24	14	37	41	4	
10	49	74	11.2	23	26	3	27	33	6	↑ Bowel management
11	120	118	- 0.9	50	50	0	54	58	4	
12	40	45	2.2	21	29	8	28	32	4	Cold-hot sensitivity, improve cought
13	82	101	8.5	50	50	0	34	43	9	↑ Bowel management
14	116	116	0	50	50	0	58	61	3	

ASIA – American Spinal Injury Association; SCIM – spinal cord independence measure. ↑ improvement.

In relation to biochemical changes, it has been described that GnRH, or its analogue LA, induces an increase in the expression of neuronal regeneration marker proteins such as 68 and 200 kDa neurofilaments and spinophilin in spinal cord neurons of rat *in vivo* (Calderón-Vallejo and Quintanar 2012) and *in vitro* (Quintanar et al. 2016a). Likewise, LA induces an increase in myelin basic protein expression, a principal constitutive protein of myelin in oligodendrocytes of spinal cord in a model of multiple sclerosis (Guzmán-Soto et al. 2012).

In the morphological study of spinal cord, both GnRH and LA treatment increased the number and caliber of nerve axons, and the white matter was well preserved in SCI rats (Calderón-Vallejo et al. 2015, Díaz-Galindo et al. 2015). In SCI, the plasticity of axons can be gained through processes of regeneration, where new axonal growth arises at the injury site from the cut end of an axon. Another possibility is axonal growth emerging from a spared axon adjacent to lesion site, or from a transected axon some distance from the site of transection (Hollis and Tuszynski 2011).

In relation to the functional effects of GnRH and LA, locomotion activity was evaluated in rats with SCI. The results showed a partial but significantly improvement in locomotor activity and gait related to stride length and stride speed (Calderón-Vallejo et al. 2015, Calderón-Vallejo and Quintanar 2012, Díaz-Galindo et al. 2015). Quintanar et al. (2016b) reported a case of a male patient with chronic incomplete spinal cord injury (ASIA C) at level T-9 treated with LA during 12 months. After the treatment, this patient increased the level of sensitivity in two dermatomes, as well as the mobility and strength in all the muscle groups of the lower extremity by approximately 30%. The percentage of recovery in the group of patients described in this study was lower than in the case reported previously. This difference may be due mainly to the magnitude of the lesion, since the patients included in this study have a higher degree of injury (ASIA A or B). It is possible that functional recovery due to LA treatment may be greater if the amount of spinal cord tissue preserved after the injury is greater. It would be important to carry out a subsequent study focused on identifying the extent of recovery with the treatment of LA depending on the degree of injury.

On the other hand, it has been reported that the use of LA offers an effective therapeutic modality for patients with moderate to severe functional bowel disease (Mathias et al. 1998, Wilson et al. 2007). This is in agreement with our results, since we observed an improvement in patients with SCI in 5 of the 14 cases. It has been previously shown that GnRH receptors are present in the digestive tract in other mammals (Huang et al. 2001) and it is possible that LA could activate these receptors and thus, improve intestinal motility.

This study is useful, because it includes chronic patients with SCI. Considering that all patients present a severe and chronic injury, the percentages of recovery obtained in this trial are significant since they directly impact on their daily life. Currently, few clinic treatment options exist for cases where the injury occurred several years ago; for example, trials with stem cells transplantation and removal of the scar (Young 2014).

In addition to the effects on the nervous system, it has been reported that GnRH and LA have a direct action on the modulation of the function of the immune system. Specifically, it has been described that GnRH and LA treatments significantly reduce the expression of proinflammatory cytokines (Guzmán-Soto et al. 2016, Quintanar and Guzmán-Soto 2013). Also, in rats with acute spinal cord injury, it was found that there was a decrease in activated microglia in the spinal cord of animals that received treatment with LA (Díaz-Galindo et al. 2015). Considering all previous results, we propose that this treatment protocol could also be used for patients with acute injury; however, this option is for further research. Also, other complementary ways of evaluating the recovery effects of treatment with LA could be included; such as nuclear magnetic resonance, tomography and evoked potentials before and after of LA administration.

In conclusion, treatment with LA induces improvements in sensitivity, motor activity and independence in patients with chronic SCI. The main advantage of this protocol is that it is a non-invasive method of easy and safe application, with few well described side effects and the drug already exists in commercial presentation.

ACKNOWLEDGMENTS

The authors acknowledge to Lic. Luis Ernesto Muñoz for his support in the Physical Rehabilitation Clinic of the Universidad Autónoma de Aguascalientes and Dr. Ernesto Ruiz Esparza Padilla and Ing. Victor Masaaki Ono Yoshikawa for their support in the Medical Center Medica Norte, Aguascalientes, México. To Dr. Andrés Quintanar Stephano, Dr. Javier Ventura Juárez and Dra. Marcela Villanueva Rodríguez for their medical assistance.

REFERENCES

Barrera CM, Kastin AJ, Fasold MB, Banks WA (1991) Bidirectional saturable transport of LHRH across the blood-brain barrier. Am J Physiol 261: E312–318.

Calderón-Vallejo D, Quintanar JL (2012) Gonadotropin-releasing hormone treatment improves locomotor activity, urinary function and neurofilament protein expression after spinal cord injury in ovariectomized rats. Neurosci Lett 515: 187–190.

Calderón-Vallejo D, Quintanar-Stephano A, Hernández-Jasso I, Jiménez-Hernández V, Ruiz-Ornelas J, Jiménez I, Quintanar JL (2015) Functional and structural recovery of the injured spinal cord in rats treated with gonadotropin-releasing hormone. Neurochem Res 40: 455–462.

Catz A, Itzkovich M, Agranov E, Ring H, Tamir A (1997) SCIM-spinal cord independence measure: a new disability scale for patients with spinal cord lesions. Spinal Cord 35: 850–856.

Díaz-Galindo MC, Gómez-González B, Salinas E, Calderón-Vallejo D, Hernández-Jasso I, Bautista E, Quintanar JL (2015) Leuprolide acetate induces structural and functional recovery of injured spinal cord in rats. Neural Regen Res 10: 1819–1824.

Ditunno JF, Formal CS (1994) Chronic spinal cord injury. N Engl J Med 330: 550–556.

Ditunno JF, Young W, Donovan WH, Creasey G (1994) The international standards booklet for neurological and functional classification of spinal cord injury. American Spinal Injury Association. Paraplegia 32: 70–80.

Dolan S, Evans NP, Richter TA, Nolan AM (2003) Expression of gonadotropin-releasing hormone and gonadotropin-releasing hormone receptor in sheep spinal cord. Neurosci Lett 346: 120–122.

Guzmán-Soto I, Salinas E, Hernández-Jasso I, Quintanar JL (2012) Leuprolide acetate, a GnRH agonist, improves experimental autoimmune encephalomyelitis: a possible therapy for multiple sclerosis. Neurochem Res 37: 2190–2197.

Guzmán-Soto I, Salinas E, Quintanar JL (2016) Leuprolide acetate inhibits spinal cord inflammatory response in experimental autoimmune encephalomyelitis by suppressing NF-κB activation. Neuroimmunomodulation 23: 33–40.

Harvey AR, Lovett SJ, Majda BT, Yoon JH, Wheeler LPG, Hodgetts SI (2015) Neurotrophic factors for spinal cord repair: Which, where, how and when to apply, and for what period of time? Brain Res 1619: 36–71.

Hollis ER, Tuszynski MH (2011) Neurotrophins: potential therapeutic tools for the treatment of spinal cord injury. Neurotherapeutics 8: 694–703.

Huang W, Yao B, Sun L, Pu R, Wang L, Zhang R (2001) Immunohistochemical and in situ hybridization studies of gonadotropin releasing hormone (GnRH) and its receptor in rat digestive tract. Life Sci 68: 1727–1734.

Kadoya K, Tsukada S, Lu P, Coppola G, Geschwind D, Filbin MT, Tuszynski MH (2009) Combined intrinsic and extrinsic neuronal mechanisms facilitate bridging axonal regeneration one year after spinal cord injury. Neuron 64: 165–172.

Mathias JR, Clench MH, Abell TL, Koch KL, Lehman G, Robinson M, Snape WJ (1998) Effect of leuprolide acetate in treatment of abdominal pain and nausea in premenopausal women with functional bowel disease: a double-blind, placebo-controlled, randomized study. Dig Dis Sci 43: 1347–1355.

Periti P, Mazzei T, Mini E (2002) Clinical pharmacokinetics of depot leuprorelin. Clin Pharmacokinet 41: 485–504.

Pitts LH, Ross A, Chase GA, Faden AI (1995) Treatment with thyrotropin-releasing hormone (TRH) in patients with traumatic spinal cord injuries. J Neurotrauma 12: 235–243.

Quintanar JL, Calderón-Vallejo D, Hernández-Jasso I (2016a) Effects of GnRH on neurite outgrowth, neurofilament and spinophilin proteins expression in cultured spinal cord neurons of rat embryos. Neurochem 41: 2693–2698.

Quintanar JL, Díaz-Galindo MC, Calderon-Vallejo D, Hernandez-Jasso I (2016b) Clinical effect of leuprolide acetate, an agonist of GnRH, on sensitive and motor function in a patient with chronic spinal cord injury. J Neurol Res 6: 111–113.

Quintanar JL, Guzmán-Soto I. (2013) Hypothalamic neurohormones and immune responses. Front Int Neurosci 7: 1–17.

Quintanar JL, Salinas E (2008) Neurotrophic effects of GnRH on neurite outgrowth and neurofilament protein expression in cultured cerebral cortical neurons of rat embryos. Neurochem Res 33: 1051–1056.

Quintanar JL, Salinas E, González R (2007) Expression of gonadotropin-releasing hormone receptor in cerebral cortical neurons of embryos and adult rats. Neurosci Lett 411: 22–25.

Sweis R, Biller J (2017) Systemic complications of spinal cord injury. Curr Neurol Neurosci Rep 17: 8.

Varma AK, Das A, Wallace G, Barry J, Vertegel AA, Ray SK, Banik NL (2013) Spinal cord injury: a review of current therapy, future treatments, and basic science frontiers. Neurochem Res 38: 895–905.

Wilson AC, Meethal SV, Bowen RL, Atwood CS (2007) Leuprolide acetate: a drug of diverse clinical applications. Expert Opin Investig Drugs 16: 1851–1863.

Wu J-C, Huang W-C, Tsai Y-A, Chen Y-C, Cheng H (2008) Nerve repair using acidic fibroblast growth factor in human cervical spinal cord injury: a preliminary Phase I clinical study. J Neurosurg Spine 8: 208–214.

Young W (2014) Spinal cord regeneration. Cell Transplant 23: 573–611.