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THALIDOMIDE ADMINISTRATION INHIBITS THE CLINICAL EVOLUTION OF THE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE) IN LEWIS RATS: PRELIMINAR RESULTS

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Abstract

Multiple sclerosis (MS) is a chronic neurodegenerative inflammatory disease of the central nervous system. Several immunomodulatory agents have been used to prevent MS acute exacerbations. Tumor Necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) are two major inflammatory mediators. Recently, we investigated in our laboratory the therapeutic value of thalidomide, a recognized TNF-α inhibitor, for the treatment of MS using the classical Lewis rat model of experimental autoimmune encephalomyelitis (EAE). The experimental study revealed that thalidomide reduces the incidence of EAE development in 90% of the cases. Hence we hypothesized that thalidomide may be an important therapeutical tool for prevention of acute exacerbations of the MS.

Keywords

Experimental autoimmune encephalomyelitis. Thalidomide. Tumor necrosis factor alpha. Interferon gamma.

1 INTRODUCTION

Multiple sclerosis (MS) is an autoimmune disease which involves infiltration by CD4+ and CD8+ T cells in the central nervous system (CNS), characterized by localized areas with demyelination. MS is not only incurable but also seriously disabling, typically running a relapsing/remitting course which eventually becomes progressive (1,2,3).

The pathogenesis of multiple sclerosis is similar to that found in encephalomyelitis autoimmune experimental (EAE), considered an animal model of MS. EAE and MS involves infiltration by CD4+ and CD8+ T lymphocytes mediated by the expression of inflammatory cytokines (PETERSON et al., 1995; BELL et al., 2007; SASTRY et al., 1999). Tumor Necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) are two major inflammatory mediators in MS, and EAE inducing peripheral immune cells infiltrate the CNS, targeting myelin antigens, resulting in the development of inflammatory process and demyelination (MELANSON et al., 2009; RAUSCH et al., 2009; BALABANOV et al., 2006).

Nowadays, the first-line therapy for relapsing-remitting MS is represented by the use of subcutaneous glatiramer acetate and beta interferons (IFN-β). However, the debate about the prescription of beta interferons in the treatment of MS continues because of the high cost of the treatment (BELL et al., 2007). The socioeconomic burden of MS is substantial, because of the debilitating nature of this chronic and progressive disease that affects people in the most productive years of life.

Several immunomodulatory therapies have been tried to prevent the MS acute exarcebations (PETERSON et al., 1995; BELL et al., 2007). In 1995, it was demonstrated that thalidomide inhibits TNF-α production by lipopolysacharide (LPS) and liporabinomannan-stimulated human microglial cells. In 1999 the inhibition of TNF-α production with thalidomide for altering the natural history of MS was hypothesized (SASTRY et al., 1999). However, the search on the medical literature using MEDLINE revealed that this drug has not been tested in MS or EAE.

Recently, we investigated in our laboratory the therapeutic value of thalidomide for the treatment of MS using the classical Lewis rat model of EAE. Female Lewis rats (n=10) were treated with thalidomide during the course of EAE. Our data demonstrated that thalidomide reduces the incidence of EAE development in 90% of the cases.

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The data of this original study suggested that thalidomide may be a new and inexpensive therapeutic option for the treatment of MS, however further experiments must be performed to investigate this hypothesis.

2 MATERIAL AND METHODS

2.1 TEST ANIMALS

Young adult, normal female isogenic Lewis rats, with ages varying between 6 to 8 weeks, body weight in 120–150 grams, were obtained from CEMIB – UNICAMP – SP - Brazil. This project was approved by the Ethics Committee on Test Animals of the Reproduction Biology Center – *Universidade Federal de Juiz de Fora*. A total of 40 animals were used; 10 were used as negative controls and did not receive any type of treatment and they were slaughtered for standard clinical, histological and serological normal analyses. The other animals were distributed into 3 groups as described below and were killed on the 15th day after EAE induction:

a) Negative control group – 10 animals;
b) EAE group (animals injected with guinea pig macerate and complete Freund adjuvant + 4 mg *Mycobacterium tuberculosis)* – 10 animals;
c) EAE group treated subcutaneously with thalidomide 30 mg/kg body weight uninterruptedly for 15 days – 10 animals;

This project was approved by the Ethics Committee on Test Animals of the Reproduction Biology Center – Federal University of Juiz de Fora.

2.2 INDUCTION OF EAE

Guinea pig spinal cord (GPSC) and the strain of *Mycobacterium tuberculosis* (ATCC H37 RA, Difico, Detroit, Ml) used for induction of EAE were gently provided by Thereza Fonseca Quírico dos Santos, MD from Fluminense Federal University.

For preparing the complete antigenic emulsion (GPSC-CFA), the homogenization of one part of the GPSC macerate was carried out with two parts of incomplete Freund adjuvant (IFA) added by 4 mg *Mycobacterium tuberculosis* (MBT H37 RA, Difco, Detroit, Ml) per milliliter of adjuvant, thereby forming the Complete Freund Adjuvant (CFA). The emulsion was prepared at room temperature by means of aspiration and continuous ejection of the components for approximately 20 minutes. Sterile bottles, plastic syringes and 25x12 mm needles were used. The emulsion was considered ready when, upon dropping one drop in the water, the drop remained intact. The emulsion was prepared right before use (CARVALHO, 1999).

The animals were inoculated with 100 μL antigenic emulsion; the injection site was in the foot pad of back leg and marking of the groups was carried out. The animals were observed every day for the emergence of clinical signs (Table I) and they were slaughtered 15 days after inoculation.

2.3 ADMINISTRATION OF THALIDOMIDE

The rats received subcutaneous injections of thalidomide 30 mg/Kg (Grunenthal GMBH, Stolberg, Germany). Thalidomide was dissolved in dimethyl sulphoxide (DMSO) (Sigma Chemical Co., St Louis, MO) and sterile saline solution and the final DMSO concentration was 2% (AARESTRUP et al., 1995); final thalidomide concentration was 90 mg/mL.

2.4 EVALUATION OF EAE CLINICAL SIGNS

After being inoculated, the animals were observed every day for 2 hours, for 15 uninterrupted days, and the clinical signs were registered according to LEADBETTER (1998) and MOHAMED et al (2004) as shown in the table below:

Table I: Clinical score of Lewis rats with EAE.

SCORE	CLINICAL SIGNS
0	Healthy
1	Loss of tail tonus
2	Partial paralysis of back limbs
3	Severe paralysis of back limbs
4	Tetraplegia
5	Death

2.5 STATISTICAL ANALYSIS

Data will be expressed in mean ± standard deviation. Statistical analysis was performed with t- Student and Mann-Whitney tests with a significance level of $p<0.05$.

3 RESULTS AND DISCUSSION

The 40 rats used were clinically followed up during the entire experiment. The groups behaved in a different manner during the experiment. The control group used 10 rats, and in there was not any clinical neurological sign of EAE such as loss of the tail tonus (score 1), partial paralysis of the back limbs (score 2), severe paralysis of back limbs (score 3), tetraplegia and moribund animals (score 4) or death of animals (score 5).

Lewis rats inoculated with GPSC – CFA started presenting the EAE symptoms around the sixth day after inoculation. At this stage,

two animals presented flaccidity and tail atony (score 1). On the eighth day after induction all the animals already presented tail drop (score 1), but the majority of animals still presented preserved movements of front and back limbs except for an animal with partial paralysis of the back limbs (score 2). Around the 10th day after inoculation the animals were agitated and had some difficulty to move. On that day, 7 animals presented partial paralysis of back limbs (score 2) and 2 animals already presented severe paralysis of back limbs (score 3). Between the 13th and 14th test days, all the animals continued showing EAE symptoms and 7 animals presented severe paralysis of back limbs (score 3); 2 animals presented tetraplegia and moribund status (score 4) and one animal presented partial paralysis of back limbs (score 2) (Graph 1). Table 1 shows the clinical score of EAE on the day when the animals were slaughtered, i.e., on the 15th day after EAE induction.

Animals inoculated with GPSC – CFA and treated with thalidomide 30 mg/Kg subcutaneously for 15 days had a result completely different from the other groups; interestingly, only one animal (A) presented neurological symptoms of EAE which started around the 7th day with loss of tail tonus (score 1); this animal started to presenting partial paralysis of the back limbs (score 2) on the 10th post-inoculation day, and ultimately it presentedsevere paralysis of back limbs on the day of the slaughter (15th post-inoculation day) (Table 1).

Table 2 shows the clinical score presented by the 10 animals of each group on the day of the slaughter (15th post-inoculation day) and the score means presented by animals of groups EAE and EAE treated with thalidomide.

Table 2: Effect of pentoxifylline and thalidomide on the course of the clinical score per animal (n=10) with EAE on the 15th post-inoculation day. I = Control; II = EAE; III = EAE + thalidomide, Letters = animals and numbers in the table = clinical score.

	A	B	C	D	E	F	G	H	I	J	**MEAN 15th day**
I	0	0	0	0	0	0	0	0	0	0	**0**
II	3	3	3	3	4	3	4	3	3	2	**3.1**
III	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.3**

The immunomodulatory therapies used in the treatment of MS patients are associated with increased benefits compared with symptom management alone. Nowadays, the first-line therapy for relapsing-remitting MS is represented by the use of subcutaneous glatiramer acetate and beta interferons (IFN-β)(HORGA; HORGA, 2007). However, the debate about the prescription of beta interferons in the treatment of MS continues because of the high cost of the treatment (ILLES et al., 2005; BELL et al., 2007; MELANSON et al., 2009). Thalidomide is used as first line of treatment in many diseases where TNF-α cytokine is an important mediator of tissue damage (CHING et al., 1998).

4 CONCLUSIONS

Our previous results using the Lewis rat model of EAE support the hypothesis that thalidomide should prevent acute exacerbations and modify the relapsing-remitting MS course in humans. In addition, thalidomide used in MS patients may reduces the immunomodulatory therapy cost. Finally, we believed that other experimental studies must be performed to investigated the immunopathological effects of thalidomide in EAE. In addition, well conducted trials in MS patients would welcome to investigate the present hypothesis.

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