Influence of spinal cord protein hydrolysate upon the blood brain barrier changes due to experimental allergic encephalomyelitis in Lewis rats. Ultrastructural study

Barbara Kwiatkowska-Patzer1, Bożena Baranowska3, Michał Walski2, Andrzej W. Lipkowski1

1 Neuropeptide Laboratory, Medical Research Centre, Polish Academy of Sciences, Warszawa, Poland
2 Laboratory of Cell Ultrastructure, Medical Research Centre, Polish Academy of Sciences, Warszawa, Poland
3 Industrial Chemistry Research Institute, Warszawa, Poland

A specific protein (antigen) given orally is a known method of introducing tolerance of immunological response to this antigen. This method has recently been reviewed by some authors as a possible tool in the treatment of autoaggressive diseases, such as multiple sclerosis. The experimental allergic encephalomyelitis (EAE) respected animal model for MS was used for the study.

The aim of the study was the evaluation of the effect of pig spinal cord protein hydrolysate given orally upon the ultrastructural changes in the blood-brain barrier image in EAE.

Changes of EAE are as follows: opened channels from basal membrane (tight junction) on the border with astrocytes, fragments of organelles of the cells, oedema of astrocytes, presence of vesicles with fluid, presence of macrophages with phagolysosomes. After pre-treatment with spinal cord hydrolysate up to 6 weeks all the above changes were normalised.

These findings are promising as a possible tool in the clinical treatment of sclerosis multiplex.

key words: blood-brain barrier, oral tolerance, multiple sclerosis, experimental allergic encephalomyelitis, autoaggressive diseases

INTRODUCTION

The aim of this study was to determine the effect that spinal cord protein hydrolysate given orally has on blood-brain barrier (BBB) changes in experimental allergic encephalomyelitis (EAE).

EAE is the accepted animal model for multiple sclerosis, an autoimmune disease of the central nervous system.

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EAE is the accepted animal model for multiple sclerosis, an autoimmune disease of the central nervous system.

The main target antigens for the immunological system in MS are thought to be: myelin basic protein — MBP, myelin-associated glycoprotein — MAG, myelin oligodendroglial glycoproteins — MOG. Oral application of these antigens and their components in small doses suppress the immunological response.

The mechanism involved in oral tolerance is clonal deletion, clonal anergy and active suppression.

The oral tolerance method is based on the thesis that proteins are only partially hydrolysed and digested and partially different peptide fragments are crossing the jejunum-blood barrier. Hypothetically there exists a system diminishing peptide antigenecity. It is known that lymphocytes T CD8 are involved in the mechanism of suppression.
Previous experiments were done with MBP and were mildly successful [1].

Our suggestion is to evoke tolerance on the wide spectrum of antigens in spinal cord proteins hydrolysate. Suppression of immunological response diminishes clinical and histopathological signs of EAE [2, 4].

We undertook an ultrastructural study to establish if the BBB changes seen in EAE disappear after treatment of EAE with spinal cord proteins hydrolysate given orally.

**MATERIAL AND METHODS**

**Animals**

Six- to eight-week-old female Lewis rats were housed in animal care facilities and maintained on standard laboratory chow and water ad libitum.

Ten female Lewis rats (200–250 g) were used in the experiments. Two animals served as control group and eight were divided into two groups — one with EAE and the second with EAE pre-treated with oral spinal cord protein hydrolysate.

**Induction of CR-EAE**

The Lewis rats were immunised in each hind foot pad with 100 μl of solution containing guinea pig spinal cord 50% homogenate in phosphate buffered saline and complete Freund’s adjuvant containing 11 mg per 1 ml pasteurised *Mycobacterium phlei*. The animals were evaluated every other day. Scores were graded as follows: 0 — no symptoms, 1 — limp tail, 2 — hind leg weakness, 3 — hind leg weakness and incontinence, 4 — paraplegia and weight loss, 5 — death.

**Induction of oral tolerance**

The animals were fed every day for 40 days with 5 mg of pig spinal cords proteins hydrolysate (preparation by Pharm. Lab. Dr A. W. Lipkowski) using syringe with 20-gauge ball-point needle [2]. The control group received 0.5 ml of water.

**Ultramicroscopic study**

On the 40th day post inoculation the rats were anesthetised and perfused with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer. Specimens were taken from the hippocampal area and fixed in the same solution for 20 hours and post-fixed in a mixture of 1% OsO₄ [3] and 0.8% K₄FeCN₆. Ultrathin sections were analysed in Jem 1200 JEM Fx electron microscope.

Ultrastructural observations were concerned with endothelial cells, perivessels space, astrocytes and macrophages and connective tissue elements. 100 electrograms were analysed from the hippocampal area. Results were presented semiquantitatively.

**RESULTS AND DISCUSSION**

Lewis rats with experimental allergic encephalomyelitis show in the endothelial cells a number of pinocytic vesicles and opened tight junctions (Fig. 1).

In the perivascular space there was present protein-rich fluid pressing perivascular cells. Basal membrane was wider with elements of proliferation. These data indicate that in EAE synthesis of fibronectin could be increased and as a main component of basal membrane can be transferred into intracellular space.

There was also evidence of oedema of astrocytes (Fig. 2). Myelin-like bodies were seen in endothelial cells beside degenerative changes in them (Fig. 3). Under basal membrane were found some collagen fibrils (Fig. 4) as a sign of the repair process. We interpret these findings as evidence of the repair process at the border between capillaries and the surrounding brain cells. We discuss the hypothesis that the non-fibroblastic cells present in rat brain are able to synthesise collagen, which leads to a process comparable with fibrosis of parenchymal organs [3, 5].

We also observed the brain macrophages with rich lysosomal structures. Increased perivascular brain phagocytes were observed in total anoxia in a way similar to our recent study of experimental allergic encephalomyelitis (Fig. 5) [6].

In the examined animals after hydrolysate treatment none of the changes described above was observed (Table 1).

**CONCLUSION**

In experimental allergic encephalomyelitis morphological changes were observed in the blood-brain barrier.

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<th>Table 1. Ultrastructural changes in blood-brain barrier</th>
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<td>EAE</td>
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<td>Endothelium: tight junctions opened</td>
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<td>Pinocytic vesicles and damaged organelae</td>
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<td>Perivessels fluid. Astrocytes oedema</td>
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<td>Increased activity of macrophages</td>
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<td>Collagen fibrils in perivessel space</td>
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Figure 1. Pinocytic vesicles (p) and opened tight junctions (arrow head) in the endothelial cells. In the perivascular space there is a protein rich fluid (F) pressing perivascular cells. Bar 1 μm.

Figure 2. Opened tight junction. Evidence of oedema of astrocytes (OE). Bar 500 nm.
Figure 3. Degenerative changes in endothelial cell. Opened tight junction. Myelin-like body (M) inside the endothelial cytoplasm. Bar 200 nm.

Figure 4. Number of pinocytic vesicles and opened tight junctions in endothelial cells. Some collagen fibrils (C) under basal membrane (as a reparative process). To the BBB is attached the macrophage with rich lysosomal structures (L). Bar 500 nm.
**Figure 5.** After hydrolysate treatment (6 weeks). In perivascular zone we observed normal neurones (N). Bar 1 μm.

**Figure 6.** After hydrolysate treatment (6 weeks). Microglial cells (G) in vessels region. Endothelial cells are normal. Bar 1 μm.
as follows: opened tight junctions, pinocytic vesicles, oedema of astrocytes, presence of macrophages with phagolysosomes, fluid rich in protein in the perivascular space pressing on pericyte.

After 40 days of oral treatment with pig spinal cord hydrolysate all the changes described above had been diminished or normalised.

These findings give the clinical implication that pig spinal cord hydrolysate given orally could be successfully used as a tool in the treatment of multiple sclerosis in humans.

REFERENCES


