# ABSTRACT

Utevska S. V. The influence of hereditary predisposition and prenatal stress exposure on formation of susceptibility to the development of experimental autoimmune encephalomyelitis in rats. – Qualification scholarly paper: a manuscript.

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The dissertation investigates the influence of hereditary predisposition and prenatal stress on the formation of susceptibility to the development of experimental autoimmune encephalomyelitis (EAE) as an animal model of multiple sclerosis (MS) in rats depending on sex and age of EAE-inducing immunization.

Analysis of the literature revealed a number of evidence of the epidemiology and types of MS, risk factors for MS, the role of sex and glucocorticoid hormones, as well as annexin AnA1 in the development of MS. In addition, previous data on the effect of prenatal stress on receptive sensitivity to sex and glucocorticoid hormones were studied. The literature on the possibility of using EAE modeling in rats to understand the mechanisms of pathogenesis of MS, as well as age and sex characteristics of the disease was also analyzed.

The sensitivity of rats to EAE-inducing immunization was determined by the type of response to the introduction of an encephalitogenic mixture, which included allogeneic spinal cord homogenate and complete Freund's adjuvant. Animals, by type of response, were divided into “sensitive to EAE-inducing immunization” (had signs of disease during observation) and “resistant to EAE-inducing immunization” (had no signs of disease during observation). The study

was performed by EAE-inducing immunization. Prenatal stress was modeled by the effect of chronic unavoidable stress on mothers in the last third of pregnancy. EAE-inducing immunization was performed on animals of both sexes at puberty (13 weeks of age), young age (18-21 weeks of age) and adulthood (29–35 weeks of age). Sensitivity analysis to EAE induction and disease course in rats was performed by assessing the incidence of EAE, mortality, time of EAE and the degree of movement disorders. In adult animals of both sexes, whose prenatal development took place in different conditions, the relationship between the level of sex hormones and the incidence and course of EAE, as well as changes in corticosterone and its secondary messenger annexin-A1 were investigated by means of EAE-inducing immunization using the method of enzyme-linked immunosorbent assay.

We have developed a model to study the peculiarities of the course of family forms of multiple sclerosis depending on the conditions of prenatal development. The simulation consists of three stages. At the first stage, EAE-inducing immunization is performed in females. Females with different sensitivity to EAE induction were selected for the parent group. In the second stage, animals from the parent group were mated. With the help of cytological examination of vaginal smears, the day of fertilization was determined. Simulation of the action of the influencing factor was performed in females during pregnancy and during the time determined by the research task. Newborn animals were kept with their mothers for twenty-one days. In the third stage, EAE was induced in all rats from the offspring of mothers with a certain sensitivity to EAE-inducing immunization. Immunization was carried out at the age determined by the task of the study.

The research first revealed the features of the age dynamics of the incidence of EAE in rats with different conditions of prenatal development. In animals whose development took place under standard conditions, the incidence rate depended on the age of EAE-inducing immunization. Pubertal rats had significantly less EAE than young and mature animals. In animals exposed to prenatal stress, the

incidence rates in the case of induction of EAE at puberty, young and adulthood did not differ.

Also, for the first time, features of morbidity and course of EAE for each separate age group of animals depending on conditions of prenatal development were revealed. At puberty, the number of sick individuals among prenatally stressed animals was slightly higher than among animals in the comparison group. In males, prenatal development did not have a significant effect on the course of the disease. In females, prenatal stress was associated with a prolongation of time from the day of the first manifestations of the disease to the day with the maximum rates of movement disorders, which led to the formation of higher levels of maximum movement disorders.

It was found that in the case of induction of EAE at a young age, the course of the disease in animals of different sexes depended on the combined effect of conditions of prenatal development and the sensitivity of mothers to EAE-inducing immunization. The lowest risk of morbidity and mortality among young rats was determined by the combined effect of “female sex” – “maternal sensitivity to in EAE-inducing immunization” – “transferred prenatal stress”. The combined effect of factors such as “male sex” – “maternal sensitivity to EAE-inducing immunization” – “prenatal stress” in sick animals was a condition for easier disease and survival.

It was shown that in the case of EAE induction in adult rats, the lowest incidence was determined by a combined effect of factors: “male sex” – “transferred prenatal stress” – “maternal sensitivity to EAE induction”. Females with different prenatal development conditions were equally ill with EAE, but some differences in the course of the disease were observed. For females from the litter of resistant mothers, prenatal stress was a condition for reducing the severity of the disease.

An enzyme-linked immunosorbent assay (ELISA) showed an association between prenatal developmental conditions, sex hormone levels on the one hand, and the incidence and course of EAE in adult animals of different sexes on the

other. Estrogen levels in prenatally stressed males were significantly lower than in animals whose development took place under standard conditions. Among prenatally stressed animals of both sexes, resistance to EAE induction correlated with significantly higher testosterone levels. It was also found that the dynamics of corticosterone and AnA1 caused by EAE induction depended on the combined effect of rat sex, prenatal development conditions and animal sensitivity to immunization. A combination of factors such as prenatal stress and predisposition to the disease was associated in males with decreased levels of corticosterone and AnA1 after induction of EAE. In females, the main factor determining the decrease in corticosterone levels on day 10 after immunization was prenatal stress, and a decrease in AnA1 was associated with susceptibility to disease. Gender differences in the dynamics of AnA1 levels were observed in the offspring of mothers resistant to EAE inducement. In males with EAE, a decrease in AnA1 was observed in those animals that were exposed to prenatal stress. In females, prenatal stress resulted in lower AnA1 levels in both health and disease development. At the same time, regardless of the conditions of prenatal development in females there was a marked decrease in AnA1 on the 10th day of the disease.

The specificity of the action of prenatal stress as a factor influencing the formation of sensitivity to EAE-inducing immunization in rats was studied. The incidence and course of EAE in young animals from the offspring of mothers who were immunized with EAE during pregnancy and animals from the offspring of mothers exposed to chronic unavoidable stress in the last trimester of pregnancy were compared. Maternal illness during pregnancy was associated with a much higher incidence rate in offspring immunized at a young age than the effects of unavoidable stress. Conditions of prenatal development particularly affected the course of the disease in male offspring. Maternal illness during pregnancy determined the early onset and longer course of the disease in male offspring than the effects of prenatal chronic stress. In males (offspring of EAE-resistant mothers) prenatal stress was associated with a more severe course of the disease compared to males whose mothers were immunized for EAE during pregnancy and were

resistant. Therefore, prenatal stress is a specific influencing factor that determines the level of morbidity and features of EAE.

**Scientific novelty of the obtained results.** For the first time, using the model of multiple sclerosis – experimental autoimmune encephalomyelitis – the dependence of the risk of developing EAE and the severity of EAE on the combined effects of such factors us “prenatal stress”, “the age of immunization”, “sex” and “the sensitivity of mothers to EAE-inducing immunization” was revealed. It was found that in the case of induction of EAE in males at puberty, the conditions of prenatal development did not have a significant impact on the course of the disease. Whereas in females, prenatal stress was associated with a prolongation of time from the day of the first manifestations of the disease to the day with the maximum rates of movement disorders, which led to higher levels of maximum movement disorders.

It was found that in the case of induction of EAE in young animals, the course of the disease depended on the combined effect of sex, conditions of prenatal development and the sensitivity of mothers to EAE-inducing immunization. The lowest risk of morbidity and mortality among young rats was determined by the combined effect of “female sex” – “maternal sensitivity to EAE-inducing immunization” – “prenatal stress”. The combined effect of factors such as “male sex” – “maternal sensitivity to EAE-inducing immunization” – “prenatal stress” in sick animals was a condition for easier EAE and survival.

It was found that in the case of induction of EAE in adulthood, the features of the disease in females were determined by the conditions of prenatal development. EAE severity parameters such as “maximum level of movement disorders” and “number of days of illness when motor disorders are equal to or greater than 2 CI scores” were significantly lower in prenatally stressed females than in animals whose prenatal development took place under standard conditions.

For the first time, the influence of prenatal development conditions on the age dynamics of EAE incidence in rats was revealed. In animals whose development took place under standard conditions, a significantly lower incidence was observed

in animals of pubertal age compared to young and mature rats. In animals exposed to prenatal stress, the incidence rates in the case of induction of EAE at puberty, young and adulthood did not differ statistically.

For the first time, it was found an association between sensitivity to EAE-inducing immunization of prenatally stressed rats of both sexes in adulthood and low serum testosterone levels.

For the first time, it was found that the dynamics of corticosterone and AnA1 caused by the induction of EAE in adult animals depended on the combined effect of rat sex, prenatal development conditions and animal sensitivity to immunization. In males, decreased corticosterone and AnA1 levels were caused by a combined effect of prenatal stress and a predisposition to disease development. In females, the main factor leading to a decrease in corticosterone levels on day 10 after immunization was prenatal stress, and a decrease in AnA1 was associated with susceptibility to disease.

**The practical significance of the obtained results.** The results are important for an in-depth understanding of the etiology of multiple sclerosis and determining the role of prenatal stress in the formation of age and gender aspects of predisposition to the development of EAE depending on the type of sensitivity of mothers to EAE induction. The obtained data allow to form a new direction of research, which focuses on the factors that act in the prenatal period of development and lead to epigenetic modifications of the genome, which, in turn, determine the features of both EAE and MS. The animal model of induction of experimental autoimmune encephalomyelitis used by us to study the influence of conditions of prenatal ontogenesis on the formation of predisposition to development and on the course of family-associated forms of multiple sclerosis expands experimental possibilities of studying the combined influence of environmental factors and genetic background on the etiology and pathogenesis of multiple sclerosis.

The results will contribute to the development of new methods for the prevention of MS, predicting the risk of developing the disease in the offspring of

mothers with MS, predicting the course of the disease in patients with family MS and identifying new therapeutic approaches to multiple sclerosis.

**Publications.** Based on the materials of the dissertation, 2 articles were published in scientific professional journals of Ukraine; 1 article in a foreign journal indexed by international scientometric databases; 1 article in a specialized scientific journal of Ukraine. Four abstracts of reports have been published in the materials of international and Ukrainian congresses and conferences.

**Key words:** experimental autoimmune encephalomyelitis, prenatal stress, age, sex, maternal sensitivity to EAE induction, pregnancy, sex hormones, corticosterone, annexin-A1.