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Evaluation of the effect of metenkephalin and tridecactid combination on corticotropic stimulation of the adrenal gland in patients with the relapsing-remitting form of multiple sclerosis

Abstract

Objectives: The Relapsing-Remitting form of Multiple Sclerosis (RRMS) affects 85% of patients, characterized by alternating onset and remission of disease symptoms. The standard treatment for MS relapse is pulse corticosteroid therapy. In this study, the efficacy of a combination of neuropeptides (metenkephalin and tridecactid) was evaluated as an alternative to pulse corticosteroid therapy for the treatment of RRMS relapse. Tridecactide is considered to have an immunomodulatory effect, similar to α-MSH, which has been suggested to exert anti-inflammatory effects by acting through Melanocortin Receptors (MCR) in the CNS, and by activating descending anti-inflammatory pathways.

Methods: A prospective clinical study was conducted on 40 adult patients with clinically confirmed relapse of RRMS using the Mc Donald criteria according to the 2005 revision. The study evaluated the effects of the combination of neuropeptides from the test drug compared to high doses of methylprednisolone, in the treatment of RRMS relapse. Patients were randomized to two relapse treatment options. Treatment arm A included the use of a combination of metenkephalin and tridecactid, while treatment arm B involved high doses of methylprednisolone. The effect on cortisol levels were measured in both treatment arms.

Results: The study found that the combination of metenkephalin and tridecactid increased cortisol levels significantly more than methylprednisolone.

Conclusion: This suggests that the use of neuropeptides could be an effective alternative to pulse corticosteroid therapy for the treatment of RRMS relapse. Further studies are necessary to determine the long-term effects of neuropeptides in the treatment of RRMS.

Burnazović-Ristić Lejla[1]*; Maleškić Kapo S[1]; Rakanović-Todić M[1]; Aganović-Mušinović I[2]; Mačkić-Đurović M[3]; Šabanagić-Hajrić S[4]; Suljić-Mehmedika E[4]

[1]Department of Pharmacology, Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

[2]Department of Immunology, Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

[3]Department of Genetics, Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

[4]Clinic for Neurology, Clinical Center, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

***Corresponding author: Lejla Burnazovic-Ristic**
Department of Pharmacology, Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.
Tel: +387-33-217 540, Fax: +387-33-217 540;
Email: lejla.burnazovic@mf.unsa.ba

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Keywords: Multiple sclerosis; Relapsing-remitting form; Metenkephalin; Tridecactid; ACTH.

Introduction

Multiple Sclerosis (MS) is a chronic, autoimmune, degenerative disease of the Central Nervous System (CNS). The Relapsing-Remitting form of Multiple Sclerosis (RRMS) is characterized by alternating onset and remission of disease symptoms and is considered to affect 85% of patients [1]. The symptomatology of relapse can also disappear spontaneously within 2-3 weeks from the onset of symptoms. If the symptoms appear in a more pronounced form, medication treatment is necessary.

The standard in the treatment of multiple sclerosis relapse is pulse corticosteroid therapy [2]. Glucocorticoids are produced in the adrenal cortex in response to the stimulus of Adrenocorticotropic Hormone (ACTH) from the anterior lobe of the pituitary gland. To date, it is known that ACTH 1-39 (full length ACTH) and ACTH 1-24 (tetracosactide) possess corticotropic and neurotropic activities [3], which has been used in the treatment of diseases with a neuroimmunological background such as multiple sclerosis. Their long-term use can be accompanied by the characteristic side effects of excess

corticosteroids [4].

Met-enkephalin is one of the endogenous opioid peptides, while tridecactid is composed of deacetylated and deamidated α-melanocyte-stimulating hormone (α-MSH) and contains the amino acid sequence ACTH 1-13 [5]. Immunomodulatory effects have been suggested [6], while analgesic effects [7] and cytogenetic effects of the tested combination have been observed [8,9] as well as the effect of chronic use on metabolic and biochemical parameters [10,11]. Tridecactide is considered to have an immunomodulatory effect. Since tridecactid exhibits structural similarity to α-MSH, we will refer to data from the literature. α-MSH has been suggested to exert anti-inflammatory effects by directly acting on cells in the periphery and by acting through Melanocortin Receptors (MCR) in the CNS, and by activating descending anti-inflammatory pathways [12-14].

Contrary to the inhibitory effect of α-MSH in the production and action of proinflammatory mediators, it was found that α-MSH stimulates IL-10, a cytokine with a powerful anti-inflammatory effect [8,15]. Namely, in peripheral blood monocytes and monocyte cultures, α-MSH increased the production and expression of IL-10 [16]. Since IL-10 reduces the production of proinflammatory cytokines in macrophages, its concomitant regulation could result in an anti-inflammatory effect.

Like ACTH 1-39, tetracosactide also increases steroidogenesis, and its fast-acting formulation can be used to assess adrenocortical function [4]. A close interaction of neural-immune mechanisms in the pathogenesis of MS has already been implied in the literature [17] and in order to define the potentiated mechanism of action more closely, it would be interesting to investigate the influence of the combination of methenekephalin and ACTH 1-13 on the cortisol levels of MS patients.

The aim of this research is to determine the effect of MS relapse treatment with a combination of metenkephalin and tridecactide on corticotropic stimulation of the adrenal gland by measuring the level of plasma cortisol concentrations. Considering the available information on the pharmacodynamic profile of tridecactid, we started the trial from the point of view that the use of a combination of metenkephalin and tridecactid compared to the use of corticosteroid "pulse therapy" in patients with relapsing MS would significantly increase the body's hormonal response, measured by the level of cortisol.

Materials and methods

A prospective clinical study was conducted to evaluate the effects of the combination of neuropeptides from the test drug in relation to the use of high doses of methylprednisolone, in the treatment of RRMS relapse. The study of the effect on the cortisol level was carried out on blood samples from patients who were hospitalized at the Neurological Clinic of the Clinical Center of the University of Sarajevo due to disease relapse.

The research was carried out according to the principles of the current revision of the Declaration of Helsinki, in accordance with ethical standards and after obtaining the consent of the competent Ethics Committee of the Medical Faculty of the University of Sarajevo. All the subjects involved signed an informed consent for inclusion in the study before sampling. The collected samples are coded in accordance with the current regulations on the protection of confidentiality rights of research subjects.

Patients

The study included 40 adult patients, aged 18-60 years, with clinically confirmed RRMS using the Mc Donald criteria according to the 2005 revision [18]. The control group included healthy subjects (n=40).

Treatment

Patients with RRMS were randomized to two relapse treatment options. Treatment arm A (n=20) included the use of a combination of metenkephalin and tridecactid (Enkorten®) in a ratio of 1:5 according to a 21-day scheme for relapse (in week 1 for three subsequent days 3x12 mg daily; in week 2 every second day 3×12 mg daily; in week 3 every second day 3×6 mg daily). Treatment arm B (n=20) included the use of corticosteroid "pulse" therapy (methylprednisolone 1000 mg per day in a total of four doses, and continuation of oral therapy with prednisolone in doses of 100 mg during the first, then 60 mg during the second and 20 mg during the third day of treatment).

Evaluation of cortisol levels

To monitor cortisol levels, the test included the first blood sampling at the time of disease relapse (measurement 0), then sampling after one (measurement 1) and twelve hours (measurement 2) from the start of one of the treatment options, and the last sampling after the complete end of the treatment (measurement 3). In healthy volunteers in the control group (N=40), the cortisol level was measured twice, in the morning and in the evening, in order to cover the 12-hour period of normal hormonal fluctuations. Cortisol level testing in blood samples was performed using the Enzyme Immunoassay competitive technique (EIA) and the circadian rhythm of cortisol secretion and different reference values in the morning and evening hours were considered.

Statistical analysis

The statistical program SPSS 17.0 (SPSS, Inc, Chicago, Il) was used for data processing. Descriptive variables are presented through frequency distributions. After applying the Kolmogorov-Smirnov test for normality, non-parametric statistics were applied according to the distribution. Statistical testing of hypotheses was carried out in the form of pretest-posttest evaluation methodology, at a significance level of 95%. Comparability and matching of groups at baseline were tested using the General Linear Model. A non-parametric test for repeated measurements, the Wilcoxon signed rank test, was used to analyse the differences in the two pretest-post test measurements. For independent groups represented by continuous variables, the Mann-Whitney U test was used.

Results

The total number of patients included in the research was 80 subjects. The sample of MS patients was 40 subjects, and the comparison with healthy volunteers was done as a paired analysis, to ensure methodological rigidity. The subjects included were aged from 19 to 60 years. Data for 39 subjects suffering from MS were included in the statistical processing, the data from 1 subject has considered as missing.

The average age only for the affected subjects (n=39) was 39.08±11.19. By methodologically matching the control group,

the average age of the respondents included in the research (n=78) was 39.04±10.92, with no significant difference in the age distribution between the groups. In the group of MS patients, there were 43.6% male subjects (n=17) and 56.4% female subjects (n=22). Equal gender distribution was achieved by matching in the control group.

Due to the normal circadian rhythm of cortisol, each subject with MS was paired with a healthy volunteer and average cortisol values in the morning and evening hours were examined. In order to test the statistical significance, the Mann-Whitney U test for independent samples was performed and it established a statistically significant difference p=0.000 for both mentioned measurements between the group of patients (regardless of randomized therapy) and the group of healthy volunteers.

The reference values for the morning were 123-626 nmol/L, and for the afternoon 46-389 nmol/L. The average values of cortisol measurements shown clear deviations from the reference values can be observed in the therapeutic group on pulse therapy during the measurement 1 h after the applied therapy and in the evening hours.

Wilcoxon signed rank test was applied for statistical analysis of difference of cortisol values in baseline sampling and end of treatment sampling, for both treatment arms (Enkorten and pulse corticosteroid therapy). Statistical significance was not established based on the difference in the therapeutic group before and after treatment (Enkorten: Z=-.747a, p= 0.455; pulse th: Z=-.706a, p=0.480).

Discussion

The obtained results indicate a potential lack of steroidogenic potential of the combination of metenkephalin and tridecactide, which is consistent with the immunomodulatory behavior through a change in the immunological response [8,15].

Since ACTH 1-39 and ACTH 1-24 exhibit corticotropic activity [3], we wanted to examine whether ACTH 1-13 stimulates the adrenal cortex after administration. In our research, a statistically significant difference was observed between the tested drug and pulse therapy in relation to the increase in cortisol values, which was recorded by measuring 1 hour after the applied treatment and in the evening sampling.

Mean value of baseline sampling cortisol was higher for the group that was randomized to the study drug in comparison to the group that was randomized to the pulse therapy (324.58; 206.06), however, the average values of cortisol at 8 a.m. i.e. one hour after the applied therapy (1412.62; 186.25) and in the measurement at 10 p.m. (435.50; 82.70) were significantly higher in subjects who received pulse therapy. All four measurements in our research show statistically significant differences, respectively, for the applied therapy (Z=-3.248, p=<0.05; -5.301, p=<0.05; -4.942, p=<0.05; -2.691, p=<0.05).

To date, a total of five Melanocortin Receptors (MCRs), MC1R to MC5R, have been identified and the steroidogenic effects of ACTH 1-39 are thought to be mediated by MC2R activation [19]. Griffing et al. (1985) show that the application of ACTH 1-24 produces only a one-hour increase in plasma cortisol, while ACTH 1-39 and ACTH 1-18 increase both during a one-hour and a 12-hour period [20]. ACTH 1-13 probably exerts its mechanism of action through interaction with MCR. Acknowledging the fact that in our research no increase in cortisol levels beyond the reference range was observed, we believe that ACTH 1-13 does not exhibit direct steroidogenic activity due to its action via MC2R. Also, because α-MSH and similar peptides bind to MC1, MC3, MC4 and MC5, but not to the MC2 receptor, their administration does not cause side effects typical of corticotropin therapy [21].

It has been hypothesized that the neuropeptide α-MSH affects the link between the immune and neuroendocrine systems [22]. Melanocortins have the ability to stimulate the synthesis and release of many important molecules involved in inflammatory processes, including chemokines, cytokines and adhesion molecules, which results from their ability to prevent NF-κB activation [23]. MC1R is found in CNS (nerve cells), peripheral (epithelial and endothelial cells) and immune cells (monocytes, macrophages, lymphocytes, neutrophils, mast cells, astrocytes and microglia). The main mode of action of ACTH 1-13 via MC1R is the prevention of NF-κB activation, which results in the downstream regulation of the production of pro-inflammatory cytokines (IL-1, IL-6, IL-2, IL-4, IL-13) as well as the expression of costimulatory molecules (CD86, CD40, ICAM-1). For comparison, ACTH 1-13 regulates the production of IL-10, which is known to reduce pro-inflammatory cytokine production and thus directly exhibits an anti-inflammatory effect [23].

Peripheral effects of α-MSH include “anti-cytokine” action, i.e. antagonistic inhibition of the effects of certain cytokines and inhibition of their production. The mechanism of anti-inflammatory action of α-MSH is suggested through the inhibition of pro-inflammatory cytokines (TNFα and IL-1), but also by increasing the synthesis of anti-inflammatory cytokines, especially IL-10, which is confirmed by the research of Weiss et al. (1991) [24]. According to some studies, the inhibitory effects on cytokine production were significant and ranged from 30% to 50% [25,26].

The Melanocortin-2 Receptor (MC-2R), which is known as the ACTH receptor, is selectively activated by the adrenocorticotropic hormone ACTH 1-39, but not by other melanocortic peptides. This explains why α-MSH and similar peptides such as ACTH 1-13 do not stimulate steroidogenesis. Physiological effects of ACTH 1-39 on steroid production and release by the adrenal cortex, their circadian variation and stress-related fluctuation are regulated via MC2R.

ACTH (1-13) has been proven to have neuroprotective capabilities, which have been demonstrated in animal models through the ability of ACTH (1-13) to increase neural survival, improve functional recovery of sensory and motor functions, and regenerate injured parts of the nervous system in animals [27].

Furthermore, ACTH (1-13) has been shown to have antipyretic properties with the ability to control the fibrillar state without any effects on normal temperature levels [12,14,28]. Both of these functions suggest that endogenously present ACTH (1-13) contributes to the natural defence mechanisms of the host organism.

The mechanism of anti-inflammatory action of α-MSH is not fully defined [28], and given that its structure impresses as tridecactid and scientific implications are assigned to it analogously.

Conclusion

The results of the study indicate the lack of steroidogenic potential of the combination of metenkephalin and tridecactide, which is in accordance with its previously described immunomodulatory behavior.

In our research, a statistically significant difference was observed between the tested drug and pulse therapy in relation to the increase in cortisol values. One hour after the applied therapy (1412.62; 186.25) and in the measurement at 10 p.m. (435.50; 82.70) cortisol values were significantly higher in subjects who received pulse therapy.

The tested combination contained in the test drug has a positive effect on immunomodulation and increased production of IL-10, the basic anti-inflammatory cytokine.

Immunomodulation with a shift of cytokine response suggest further scientific exploration, having in mind the potential bimodal effects of the investigated combination.

Further exploration of the safety and efficacy of the neuropeptide combination in expanding the indications of autoimmune treatment is suggested, with special emphasis on Rheumatoid Arthritis, Mb Chron's and ulcerative colitis, considering the findings the supports the potential immunomodulation non-significantly impacting the cortisol levels.

Declarations

Ackowledgmenet: The manuscript is based on thesis by the author Lejla Burnazovic-Ristic [30].

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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