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Impact of methylprednisolone treatment on the expression of macrophage inflammatory protein 3α and B lymphocyte chemoattractant in serum of multiple sclerosis patients

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Abstract:
In order to extend our studies designed to elucidate the mechanism of action of intravenous methylprednisolone (*iv*MP) in symptomatic therapy of relapses in multiple sclerosis (MS) victims, we have evaluated the expression of chemokines: macrophage inflammatory protein 3α (MIP-3α) and B-lymphocyte chemoattractant (CXCL13) before and after treatment. The data from further exploration of the MP mechanism of action in MS relapses may be helpful in establishing the treatment design, that would be specific both for individuals, and for the disease phase.
The mean levels of MIP-3α in sera of MS patients showed no statistically significant differences compared to control subjects. The comparison of MIP-3α level before and after therapy with *iv*MP gave the same result. The CXCL13 expression in serum was significantly higher in the group of MS patients than in healthy subjects. After therapy with *iv*MP the estimated level demonstrated an increase as related to the initial values found in MS patients. Such a response was seen also in the responder but not in non-responder subgroup.
The enhancement of CXCL13 expression after *iv*MP therapy in MS relapses may explain the lack of a long-term effect of MP therapy in MS. The observed difference in CXCL13 expression between responder and non-responder group of patients should be regarded as a step towards elucidation of the therapeutic effect of *iv*MP in MS relapses.

Key words:
methylprednisolone, multiple sclerosis, CXCL13, MIP-3α

Abbreviations: CXCL13 – B-lymphocyte chemoattractant, CSF – cerebrospinal fluid, CXCL10 – interferon γ-inducible protein, EAE – autoimmune encephalomyelitis, EDSS – expanded disability status scale, *iv*MP – intravenous methylprednisolone, MIP-3α – human macrophage inflammatory protein 3α, MP – methylprednisolone, MS – multiple sclerosis

Introduction

Despite great progress in immunomodulatory treatment of multiple sclerosis (MS), which improves the natural course of the disease, the symptomatic treat-

ment of MS relapses remains still of significant importance. A generally accepted method is the intravenous methylprednisolone (*iv*MP) treatment. *iv*MP hastens the recovery from attacks of MS in many, though not in all MS victims. MP acts by affecting various immunological events involved in the pathological process of MS [1, 5, 7, 14–16]. In our previous studies [13], we have established that *iv*MP diminishes the elevated expression of interferon γ-inducible protein (CXCL10), one of many chemoattractants for activated T cells, and this effect seems to be an important factor in the mechanism of MP action on MS relapses. Another not well understood aspect of MP treatment is the effect exerted on both cytokines targeting B-cells as well as on macrophages [2]. It is clear now that also activated B-cells and not only T-cells are involved in the immunological mechanism of MS.

The B-lymphocyte chemoattractant (CXCL13) is critical for the secondary development of lymphoid tissue and navigation of lymphocytes within compartments of other tissues. Recent studies presented by Krumbholz et al. [9] have demonstrated that CXCL13 is produced in actively demyelinating MS lesions, but not in chronic inactive plaques. However, the role of this chemokine in pathological events connected with MP action in MS relapses is obscure.

Macrophage inflammatory protein 3α (MIP-3α) has an impact not only on macrophages but also on B-cells, which are highly responsive to this chemokine [10], MIP-3α plays also a critical role in the sensitization phase of autoimmune encephalomyelitis (EAE), an animal model imitating MS [4].

The contribution of data from further elucidation of the MP mechanism of action in MS relapses may not only be helpful in establishing the treatment design, which would be specific both for individuals and for the disease phase, but that also it would pave the way for better understanding of the immunological mechanism of MS attacks.

Materials and Methods

Peripheral blood was collected from 30 patients (20 females and 10 males) with clinically definite MS according to criteria of Mc Donald et al. [13], one day before the onset of MP treatment and five days after completion of the MP treatment. Twenty healthy volunteers donated blood samples to make up the control group. The blood was collected at 9.00 a.m. ± 10 min. On the day of blood collection the patients and control subjects abstained from any alcohol intake and from tobacco smoking. Intravenous administration of MP (Solu-Medrol, Belgium) at a dose of 1.0 g, over a period of five consecutive days was used to treat a new relapse of the disease, during which the pyramidal and cerebellar systems were affected the most. The MS patients were in the relapsing-remitting phase of the disease. None of the patients was in the secondary progressive course of MS and none had other comorbid diseases. Neither of them was treated with other medicaments. Female patients had to refrain from intake of hormonal contraceptives. Among participants of the study, female patients were aged from 22 to 47 years (mean 35.8 years) and male patients were aged from 25 to 50 years (mean 37.2 years). The duration of MS was 7.5 years on the average, ranging from 2 to 20 years. The mean of expanded disability status scale (EDSS) at the time before start of the MP treatment was 2.8 (0.5 to 5.0). Depending on the observed clinical effect of MP treatment, the patients were divided into two subgroups: the responders (marked improvement, 15 cases) and the non-responders (those showing only minimal or no amelioration, 15 cases). The categorization of MS patients into the responder or non-responders subgroups was based on Kurtzke EDSS. The patients were included to the responder group when the decrease in EDSS score was equal or greater than 1.0 point, two weeks after conclusion of the therapy. The patients of non-responder subgroup manifested a decrease in EDSS score of 0.5 point or 0.0. The control group comprised 20 healthy adults (14 females and 6 males), aged 25 to 46 years (mean of 36.4 years).

The study was approved by the Ethics Committee of the University of Medical Sciences in Poznań.

The levels of MIP-3α and CXCL13 in blood serum were measured in duplicate using the ELISA immunoassay tests of Quantikine human MIP-3α and CXCL13 kits; (R&D Systems, USA).

For statistical evaluation of differences between MS and control subjects, the nonparametric U-Mann-Whitney test for two independent samples was used. Data obtained from the MS groups before and after *iv*MP therapy were evaluated by the Wilcoxon test for paired variables.

Results

The mean level of MIP-3α in sera of MS patients compared to those established for control subjects showed no statistically significant differences. The comparison of MIP-3α level before and after a 5-day treatment with *iv*MP (Tab. 1, Fig. 1) gave the same result. The observed differences between responders and non-responders were also nonsignificant (Tab. 3).

The serum concentration of CXCL13 was significantly higher in the group of MS patients in comparison to healthy subjects. After therapy with *iv*MP the estimated level was not only higher than in the control group but it also demonstrated an increase in relation to the initial values found in MS patients (Tab. 2, Fig. 2). The same observation applied to the responder subgroup, which demonstrated a marked increase in CXCL13 in sera of MS patients after *iv*MP therapy. Such response, however, was not seen in the non-responder subgroup. The differences between responder and non-responder subgroups were statistically significant (Tab. 4).

Tab. 1. The effect of treatment with methylprednisolone (Solu-Medrol) on the serum level of MIP-3α (pq/mL) in MS patients

	Control group n = 20	MS patients before treatment n = 30	MS patients after 5-day treatment n = 30
Mean	7.40	14.77	24.23
SD	5.40	34.23	76.37
Median	6.99	5.17	4.87
Min	0.70	0.96	0.83
Max	21.57	145.46	404.20
Statistical significance p (Mann-Whitney test)	Control *vs.* MS cases before treatment 0.498		MS cases before *vs.* after treatment 0.611

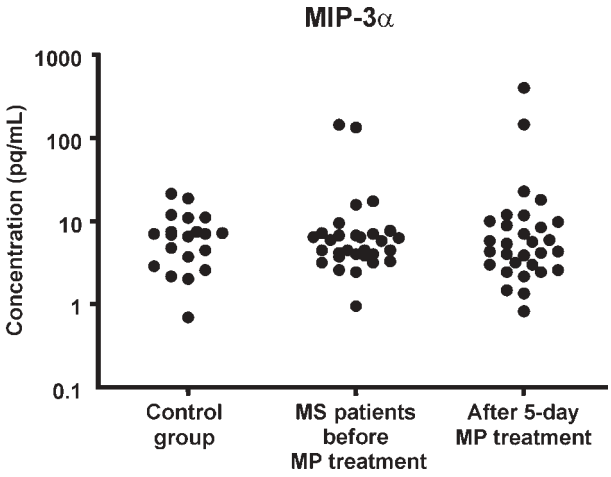

Fig. 1. MIP-3α in serum of MS patients

Tab. 2. The effect of treatment with methylprednisolone (Solu-Medrol) on the serum level of CXCL13 (pq/mL) in MS patients

	Control group n = 20	MS patients before treatment n = 30	MS patients after 5-day treatment n = 30
Mean	29.7	59.2	74.6
SD	39.6	46.0	49.9
Median	14.4	47.2	59.5
Min	6.5	18.9	21.3
Max	151.3	229.3	262.5
Statistical significance p (Mann-Whitney test)	Control *vs.* MS cases before treatment 0.000014*		MS cases before *vs.* after treatment 0.042776*

* p – statistical significance

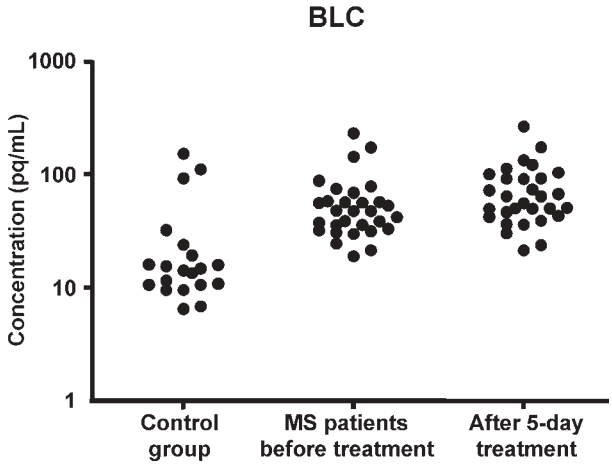

Fig. 2. CXCL13 in serum of MS patients

Tab. 3. The effect of treatment with methylprednisolone (Solu-Medrol) on the serum level of MIP-3α (pq/mL) in MS patients divided into responder and non-responder groups

	Control group n = 20	MS patients before treatment (responders) n = 15	MS patients after 5-day treatment (responders) n = 15
Mean	7.40	6.96	7.13
SD	5.40	4.50	5.65
Median	6.99	6.46	5.85
Min	0.70	0.96	0.83
Max	21.57	17.54	22.92
	Control group n = 20	MS patients before treatment (non-responders) n = 15	MS patients after 5-day treatment (non-responders) n = 15
Mean	7.40	22.67	41.32
SD	5.40	47.67	106.88
Median	6.99	4.49	4.35
Min	0.70	3.18	1.49
Max	21.57	145.46	404.20
Statistical significance p (Wilcoxon test)	Responders before treatment *vs.* after treatment 0.826		Non-responders before treatment *vs.* after treatment 0.496

Tab. 4. The effect of treatment with methylprednisolone (Solu-Medrol) on the serum level of CXCL13 (pq/mL) in MS patients divided into responder and non-responder groups

	Control group n = 20	MS patients before treatment (responder) n = 15	MS patients after 5-days treatment (responder) n = 15
Mean	29.7	50.5	83.6
SD	39.6	36.4	65.1
Median	14.4	38.7	63.6
Min	6.5	18.9	21.3
Max	151.3	171.5	262.5
	Control group n = 20	MS patients before treatment (non-responder) n = 15	MS patients after 5-days treatment (non-responder) n = 15
Mean	29.7	67.9	65.0
SD	39.6	53.8	27.5
Median	14.4	47.2	55.3
Min	6.5	21.3	23.7
Max	151.3	229.3	120.8
Statistical significance p (Wilcoxon test)	Responders before treatment *vs.* after treatment 0.036		Non-responders before treatment *vs.* after treatment 0.427

Discussion

The imbalance between pro- and anti-inflammatory cytokines seems to be a significant factor in the ups and downs of relapses, i.e. in the most frequent relapsing-remitting forms of MS. MP plays an important part in the treatment of acute phases of the disease. Intravenous administration of MP has been shown to hasten the recovery from relapses but it has failed to exert any notable impact on the long-term evolution of MS [3, 4, 11, 12].

MIP-3α is a major chemoattractant for leukocytes to sites of antigen challenge, thus functioning as a natural ligand for monocyte chemotactic protein-1 (MCP-1) receptor [6, 8, 10]. Our recent studies have revealed a marked, though statistically nonsignificant increase in MIP-3α expression in MS patients both before and after *iv*MP therapy.

Our finding concerning the enhancement of CXCL13 expression after *iv*MP therapy in MS relapses seems to be more interesting. CXCL13 is known to act as a regulator of B cell migration in lymphoid tissue as well as in actively demyelinating MS lesions [9, 12]. Wang et al. [17] have emphasized the existence of a strong linkage of CXCL13 to immune cells which, consequently, affects the immunoglobulin level in cerebrospinal fluid (CSF). These observations may explain the lack of a long-term effect of MP therapy in MS and the well-known fact that even when MS is clinically silent, an inflammatory reaction in CSF may develop and be observed (increase in CSF cell count and the presence of oligoclonal bands, as indicative of intrathecal Ig G synthesis).

Another important observation that has been made involves the diversity of reactions demonstrated by MS patients subjected to therapy of relapses by means of *iv*MP. Hence, it has proven possible and desirable to distinguish two subgroups of patients, i.e. responders and non-responders to *iv*MP therapy. Wang et al. [17] found that the early clinical improvement was associated with a decrease in CSF CD4+, CD29+ helper inducer T cells. Such changes, however, have been practically absent in patients who have shown no clinical improvement. Other subsets of lymphocytes, CSF albumin or immunoglobulin gamma (Ig γ) levels have manifested no differences between responder and non-responder groups. Our studies have revealed a difference in CXCL13 expression between responder and non-responder groups of patients, when the two subgroups were analyzed separately. However, it would be only a speculation to discuss the obtained results as a step towards elucidation of the therapeutic effect of *iv*MP in MS relapses. The open question is also if CXCL13 neutralizing agents or CXCL13 receptor antagonists might be applied to treat active MS.

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