The Effect of Therapeutic Plasma Exchange on the Bioavailability of Interferon Beta. Pilot Study

N. Giedraitiene*
R. Kizlaitiene*
V. Budrys*
G. Kaubrys*
L. Griskevicius**
V. Valceckiene**
M. Stoskus**
A. Griskevicius**
J. Audzijoniene**

**Clinics of Neurology and Neurosurgery, Faculty of Medicine, Vilnius University; Center of Neurology, Vilnius University Hospital Santariskiu Clinics, Lithuania*

***Center of Hematology, Oncology and Transfusion Medicine, Vilnius University Hospital Santariskiu Clinics, Lithuania*

Abstract. *Background and purpose.* The development of neutralizing antibodies (NAbs) against interferon beta (IFN-) during IFN- treatment in multiple sclerosis (MS) patients has been a significant clinical problem. Persistent, high-titer NAbs-IFN- reduce or eliminate biological activity of IFN- therapies for MS and are associated with reduction in efficacy. Therapeutic plasma exchange (TPE) removes the circulating antibodies that are thought to be active in the diseases, so we hypothesized that TPE can restore the ability of IFN- to induce the Myxovirus Resistance Protein A (MxA) mRNA expression and the maintenance plasmapheresis can sustain the bioavailability of IFN- .

Methods. Eligible patients underwent primarily four separate plasma exchange sessions and after the induction TPE sessions they were transferred to the maintenance plasmapheresis. Bioactivity of interferon beta was expressed as *in vivo* MxA mRNA induction in whole blood using real time PCR.

Results. Six patients with RRMS and low IFN- bioavailability detected by the MxA mRNA response were included. Four patients after induction plasmapheresis became biological responders. In two patients an increase of MxA mRNA expression was found, but the values persisted below the cut-off and the patients remained as "poor biological responders". The effect of maintenance plasmapheresis was quite transient: MxA mRNA expression values reverted to the baseline levels after one or two months.

Conclusion. Plasma exchange may restore the bioavailability of IFN- in some patients, but the effect of maintenance plasmapheresis on the bioavailability of IFN- is transient.

Keywords: interferon- , neutralizing antibodies, interferon- bioactivity, MxA mRNA expression, therapeutic plasma exchange.

Neurologijos seminarai 2013; 17(58): 288–296

INTRODUCTION

Interferon beta (IFN-) has been shown to be a safe and effective treatment for relapsing-remitting multiple sclerosis (RRMS) and is widely used as a first-line treatment. IFN- balances the expression of pro- and anti-inflammatory agents in the brain, and reduces the number of inflammatory cells that cross the blood brain barrier. Through these mechanisms IFN- achieves its antiproliferative, anti-inflammatory and immunomodulatory effects [1, 2]. However, IFN- preparations, like other protein-based biopharmaceuticals produced by recombinant gene technologies are potentially immunogenic. Long-term use of IFN- can lead to an immune response directed against the drug that is mainly based on breaking B-cell tolerance [3]. It is clear that IFN- preparations are associated with the development of two different classes of antibodies against IFN- – binding (BAbs) and neutralizing antibodies (NAbs). BAbs bind to the IFN- molecule and may or may not interfere with its functions. NAbs interfere with functions of the IFN- molecule *in vitro*, most likely by preventing binding of IFN- to the IFN receptor on cells used in the assay and, thereby, inhibiting the functional activation of the receptor. BAbs can be demonstrated in the majority of patients treated with an IFN- preparation, but only a smaller proportion of patients develop NAbs [3, 4].

Clinical implications of neutralizing antibodies. There is emerging agreement that persistent, high-titer NAbs reduce or eliminate the biological activity of IFN- therapies for MS and are associated with reduction in efficacy. The majority of studies longer than 2 years in duration reported a higher attack rate in NAb-positive compared to NAb-negative patients [5–7]. Standard therapy with INF- reduces relapse rates in NAb-positive patients by about 30–35%, and in NAb-negative the annual relapse rate decreases up to 50% [8, 9]. Also, it has been shown in numerous trials that patients who become antibody positive have increased disease activity, measured on the brain MRI as gadolinium positive lesions or new T2-lesions, or MRI disease severity measured as T2-lesion load [10–12]. None of the pivotal trials in RRMS showed an effect of

Adresas:
Natasa Giedraitiene
Center of Neurology,
Vilnius University Hospital Santariskiu Clinics
Santariskiu str. 2, LT-08661 Vilnius, Lithuania
Tel. (3705) 2365220, e-mail: natasa.giedraitiene@gmail.com

NAbs on disease progression and neither did any of the trials in secondary progressive MS [13–16]. However, all the trials were underpowered to show an effect of NAbs because IFN- by itself had no or only marginal effect on disease progression. Also the Danish study showed only a trend toward more progression in NAb-positive patients [9].

IFN- immunogenicity. The immunogenicity of IFN- is dependent on a number of factors. These are product, treatment and patient related factors. It is generally agreed that the frequency and the amount of NAbs is significantly less in patients treated with IFN- -1a compared to patients treated with IFN- -1b [17–19] and it seems clear that IFN- -1a administered IM is less immunogenic than IFN- -1a administered SC [18, 20, 21].

Occurrence and disappearance of anti-IFN- antibodies. Low concentrations of NAbs can be detected *in vitro* with sensitive assay after six months, whereas clinically relevant NAbs usually develop between 9 and 18 months after start of IFN- therapy, and if NAbs have not developed by this time they are unlikely to develop in the future [4]. Studies of the natural history of NAbs in IFN- treated patients suggest that the NAb-positive state is often transient. Additionally, some patients revert from Nab-positive to Nab-negative status over time. Reversion is more likely when NAbs titer is less than 100–200 NU/ml, whereas patients with titers above 500 NU/ml rarely become Nab-negative within a time span of 2–3 years. Traditionally, a titer of 20 NU/ml, measured with the Kawade titration method (TRU/ml = ten times reduction units per ml), has been used as cut-off for NAb positivity [4, 22, 23].

Data from Nab-positive patients who discontinued therapy indicate that NAbs may persist for long periods after cessation of treatment [24, 25]. The longest post-treatment follow-up during which a patient maintained NAb positivity was 59 months. Only few patients reverted to the NAb negative status and in some patients the NAb titer even increased after cessation of IFN- therapy [24]. The reason for the maintenance of high NAb titers after cessation of IFN- therapy is mainly unknown. One explanation could be long-lived plasma cells. Another possible explanation is that recombinant IFN- cross-react with wild-type IFN- , and it is therefore conceivable that NAb-producing B-lymphocytes are activated by intermittently produced natural IFN- , e.g., during viral infections [4].

Measurements of neutralizing antibodies. A number of methods have been developed for the measurement of NAbs and all are based on measuring the i*n vivo* responses of IFN- -sensitive human cell lines to the application of IFN- . Binding of IFN- to the IFN receptor complex on the cells leads to a change in the expression levels of many genes, including those which have antiviral, antiproliferative and immunological properties. In the presence of NAbs these changes are inhibited.

The cytopathic effect assay. The cytopathic effect (CPE) assay is considered the gold standard method for measuring NAbs. Virus susceptible cells are incubated with IFN- and patient serum for 12–24 h and virus added. After a further 24 h, viable cells (protected by IFN- -stimulated anti-viral factors) are quantified. The NAb titer is calculated using the Kawade method. The assay is prone to variation, very time-consuming and not specific (other factors within the serum may also have anti-viral properties) [4, 26].

Assays based on the quantification of MxA. One of the major proteins induced by IFN- is MxA. The CPE assay has been modified by measuring the amount of MxA protein or MxA mRNA produced following stimulation with IFN- , in the presence of patient serum, rather than cell viability following treatment with a virus. Stimulation with IFN- leads to a dose-dependent increase in MxA protein and MxA mRNA [27]. Quantification of the MxA protein has been performed by ELISA, chemiluminescence and by fluorescence activated cell sorting. Measuring MxA production has the advantage of being faster; however, assay variability remains quite high. Extraction of MxA mRNA followed by reverse transcription and quantification by real-time PCR, using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a housekeeping gene, has been performed to measure the amount of MxA mRNA produced [26, 28]. The assay is faster than the CPE assay and is much more reliable and reproducible than both the CPE assay and MxA protein assay, but its relatively high cost may limit its adoption as a routine assay in clinical laboratories [26].

Assays based on the in vivo induction of MxA. Treatment of patients with IFN- , as described above, leads to the production of the biomarker MxA and, in the presence of NAbs, this response is lost [29–31]. Maximal MxA mRNA concentrations are achieved about 12 h after the dose of IFN- is given. To assess IFN- activity, blood samples are collected 12 h post-IFN- injection into tubes designed to preserve mRNA. The mRNA is extracted and reverse transcription is used to produce cDNA. This is quantified by real-time PCR, and MxA expression values normalized by reference to GAPDH. Results are reliable and reproducible; however, costs are relatively high [26].

An assay utilizing a cell line containing a luciferase reporter gene. The assay uses a genetically engineered human fibrosarcoma HT 1080 cell line (clone HL116) [32, 33]. The HT1080 cell line was stably transfected with the firefly luciferase gene under transcriptional control of the IFN-stimulated response element. Binding of IFN- to its receptor leads to activation of the reporter and luciferase is expressed. Addition of the luciferase substrate luciferin to cell lysates produces chemiluminescence. In the assay, patient serum is pre-incubated with IFN- for 1 h, added to HL116 cells and further incubated for 6 h. Luciferin substrate is added and the plate read in a luminescence plate reader. In the presence of NAbs, luciferase production is inhibited, and the titer quantified using the Kawade method. The assay is reliable, reproducible and relatively simple and can be completed in a day [26].

Effect of neutralizing antibodies on the bioactivity of IFN- . As it was described earlier it is clear that in

NAb-positive patients, especially those with high titers, the MxA response decreases to baseline levels indicating that NAbs abrogate the bioactivity of IFN- [34]. Thus, a good (negative) correlation between MxA mRNA expression/induction and NAbs titers was found in series of studies [30, 35–37]. Using an *in vitro* MxA induction assay to measure NAbs and mRNA MxA assessment of IFN- bioactivity, Sominanda et al. found that patients with NAbs titers of up to 150 TRU/ml still had retained IFN- bioactivity, whereas profoundly reduced levels of IFN- bioactivity were found in patients with NAbs of 150–600 TRU/ml, and titers above 600 TRU/ml were associated with loss of IFN- bioactivity [38].

Treatments and attempts to prevent or reduce the neutralizing antibodies. When NAbs have developed it is difficult to make patients revert to a NAb-negative state. Data suggested that increasing the dosage of IFN- -1b decreased or reverted the NAb titer to negative in NAb-positive patients in accordance with the hypothesis that higher frequent dosage might lead to suppression of NAbs [39].

Pulse methylprednisolone therapy may reduce the risk of developing NAbs (but possibly not high-titer NAbs of clinical importance) 12 months after start of IFN- therapy if given together with IFN- from the beginning of the treatment [40, 41]. However current evidence suggests that treatment with monthly cycles of high-dose methylprednisolone does not restore IFN- biologic response and reduce NAbs levels in established NAB-positive MS patients, who discontinue IFN- therapy [41–43] and neither did a combination of azathioprine and monthly methylprednisolone cycles in established NAb-positive MS patients [44].

Therapeutic plasma exchange (TPE), complication rates. TPE, also known as plasmapheresis, is a procedure that involves separating the blood, exchanging the plasma (typically with donor plasma or albumin solution), and returning the other components, primarily red blood cells, to the patient. TPE is a successful method for treating of many autoimmune diseases, because it removes the circulating antibodies that are thought to be active in these diseases [45].

TPE is an effective, relatively safe and well tollerated treatment for many neurological autoimmune diseases. The frequency of complications associated with TPE reported in the literature is variable and is dependent upon what is or is not considered a reaction or an expected physiologic response [45, 46]. In general, most large series of TPE report low total complication rates (5–12%) [46–49]. The adverse reactions are substantially more common with fresh frozen plasma than with albumin replacement. The most common symptoms include: 1) perioral and digital paresthesias due to hypocalcemia, 2) hypotension, muscle cramps and headaches caused by hypovolemia, and 3) urticaria and rigors related to anaphylactoid reactions to plasma. The reported overall incidence of these common symptoms is less than 10%. More serious complications such as severe anaphylactoid reactions or transfusion-related acute lung injury can follow the administration of FFP and other plasma-containing replacement fluid or can rarely occur in patients receiving treatment with ACE inhibitors receiving albumin as the replacement fluid. More serious complications have been reported in approximately 1.5% of TPE procedures [45, 50].

The effect of TPE on the IFN- bioavailability. Plasmapheresis, as far as intravenous immunoglobulins, might be considered as possible procedures to diminish NAbs generation and to restore the bioavailability of IFN- . However, they do not affect memory plasma cells. The treatment may be useful in eliminating circulating NAbs, but it should not be expected to impede the production of NAbs once it has been triggered [4, 51]. However it is still unknown whether TPE may promote recovery of IFN- bioavailability.

Three questions arise regarding NAbs against IFN- and abolished IFN- bioavailability when TPE is used in MS patients treated with IFN- . Firstly, can the TPE reduce the titer of NAbs against IFN- and restore the ability of IFN- to induce the MxA mRNA expression in general? Secondly, if the TPE restore the bioavailability of IFN- , is it possible to sustain recovered IFN- bioavailability by maintenance plasmapheresis? And thirdly, how quickly do the markers of IFN- bioavailability get back to the baseline levels after the TPE use? To answer these questions, the effect of TPE on the ability of IFN- to induce the MxA mRNA was evaluated.

MATERIALS AND METHODS

Study design. An open-label, single-center pilot study was initiated to assess the feasibility and efficacy of TPE in improving the markers of IFN- bioavailability and to evaluate the safety and tolerability of TPE in relapsing remitting (RR) MS patients when donor plasma is used for plasma replacement. 6 patients were included in the study. The study was ended earlier than planned initially because the results were so evident that it was considered unethical to continue the pilot study.

Participants. The study was performed at the Neurology and Neurosurgery Clinic, Faculty of Medicine at Vilnius University during the period 2012–2013. The study protocol was approved by the Lithuanian Bioethics Committee. All participants of the study had to sign an informed consent before the start of the study procedures. The participants of the study were MS patients treated in Vilnius University Hospital Santariskiu Clinics. The inclusion criteria for all patients were: 1) Subjects aged 18–55 years, clinically definite MS, diagnosed according to the McDonald criteria (2010 Revisions), RR MS course and EDSS score of 0.0 up to and including 5.5, 2) treatment with high-doses of IFN- ; all participants were treated with IFN- -1b (Betaferon; Schering, Berlin, Germany) 250 g every other day subcutaneously for more than 18 months, 3) no IFN- bioactivity detected by means of *in vivo* MxA mRNA response 9–12 hours after IFN- injection at screening. High BAbs titers were evaluated before MxA mRNA assay. All

patients included were clinically stable and steroids free at least three months preceding the enrolment. Key exclusion criteria included: 1) medical conditions or laboratory abnormalities that might be negatively influenced by plasma exchange sessions, for example, immune compromise, malignancy and so on, 2) severe allergic/anaphylactic reactions, in particular if a history of prior reaction to blood products existed, or any known drug hypersensitivity, 3) hypotension, and/or 4) limited vascular access.

Treatment. Eligible participants continued every other day IFN- -1b (Betaferon) 250 g subcutaneously during all the study. Five of the six patients underwent four separate plasma exchange sessions of 2.0–2.5 plasma volumes and donor plasma was used for plasma replacement. Each TPE session took 2.5–3.0 h and the sessions occurred over a 5–8 day span. One patient after the first incomplete plasma exchange session (1100 ml plasma volume was replaced) was switched to the centrifugal plasmapheresis due to the adverse reactions to the donor plasma. The patient underwent six centrifugal plasmapheresis sessions of 310–380 ml volumes every day. Five patients (except one patient who underwent centrifugal plasmapheresis) after the induction plasma exchange session were transferred to the maintenance plasmapheresis: the patients underwent one plasma exchange procedure of 1.8–2.2 plasma volume per month in order to sustain the bioavailability of IFN- . A total of three maintenance plasmapeheresis were performed (Table 1). Premedication with clemastine IM and dexamethasone IV before TPE was given to avoid hypersensitivity reactions and calcium gluconate was infused IV during the TPE to avoid hypocalcemic symptoms.

IFN- bioactivity measurement. Blood from MS patients was drawn into PAXgene Blood RNA Tube (PreAnalytiX GmbH, Hombrechtikon, Switzerland) before and 12 hours after injection of INF- . Total RNA was extracted using Purlink FFPE Total RNA Isolaton Kit (Life Technologies, Carlsbad CA, USA) according to the manufacturer's protocol. Total RNA was subsequently reverse-trascribed to cDNA using RevertAid™ M-MULV reverse transcriptase and random hexamers (Thermo Fisher Scientific, Vilnius, Lithuania). Previously published sequences for *MxA* and *GAPDH* primers and probes were used in duplex qPCR reactions [52]. qPCR reactions were performed in a 20 l reaction volume containing Maxima® Probe qPCR Master Mix (Thermo Fisher Scientific, Vilnius, Lithuania), 300 nmol/l final concentration of each primer, 200 nmol/l of each probe and 2 l cDNA solution. Uracil-DNA glycosylase (Thermo Fisher Scientific, Vilnius, Lithuania) was added into each reaction mix (0·4 units) to prevent qPCR cross-contamination with PCR products. qPCR was performed on ROTORGENE 6000 (Qiagen, Hilden, Germany) using the following cycling conditions: 2 min at 50 C followed by 5 min at 95 C and 45 amplification cycles at 95 C for 20 s, 60 C for 15 s and 72 C for 20 s. Relative quantification of MxA expression was calculated by the Cq method. A pool of healthy donors peripheral blood cDNA was used as a calibrator in Cq calculations and was run in parallel with patient samples. This calibrator was assigned the normalization ratio 1. MS patients samples before and after INF- injection were always analyzed in the same qPCR run. Two cut-off values for INF- bioactivity were determined based on MxA expression results of 30 untreated MS patients as described [53]. If values from a patient samples reached both threshold levels the patient was called "a biological responder". In case only one threshold was reached a patient was assigned to a group of "poor biological responder". If none of the two thresholds were reached a patient was called "a biological non-responder". MxA mRNA expression cut-off of <0.586 before INF- injection and MxA mRNA expression cut-off of <3.84 after INF- injection were considered as negative.

Table 1. **Assessment scheme for induction and maintenance TPE.**

	Screening	**Induction TPE**					**Maintenance TPE**			
Day/Month	0	1D	2D	3D	4D	5D	1M	2M	3M	4M
Informed consent	x									
In/Exclusion criteria	x									
Physical exam*	x	x	x	x	x	x	x	x	x	x
Neurological exam**	x					x	x	x	x	x
IFN- BAbs***	x					x	x			x
Lab values****	x						x	x	x	
IFN- bioavailability*****	x					x	x	x	x	x
TPE		x	x	x	x		x	x	x	
(S)AEs, TPE related	x	Whenever (serious) adverse events occur								

*ABP, HR, Resp, body temperature, ECG
**Neurological examination, EDSS
***BAbs measured with ELISA before MxA expression
****Hb, WBC, PLT, electrolytes, TP, coagulation (APTT, PA, fibrinogen)
*****MxA mRNA expression (before IFN- injection and 12 hours after injection)

BAbs – binding antibodies against INF- ; MxA – myxovirus resistance protein A; mRNA – messenger RNA; TPE – therapeutic plasma exchange.

RESULTS

Six patients met the inclusion criteria and were included over a 4-month recruitment period. The mean age of these patients at the screening onset was 45 years (range, 35–54 years). Four patients were women (66.7%) and two patients – men (33.3%). The average treatment duration of immunomodulatory therapy prior the screening was 3.66 (±1.86) years. The mean disability score was 3.58 (±0.86) (on the Expanded Disability Status Scale, EDSS). The mean BAbs titer in all patients was 528 BTU (±354.6 BTU). All patients were clinically stable during all the study.

Five patients underwent four separate plasma exchange sessions and one patient after first incomplete session underwent six centrifugal plasmapheresis sessions due to the side effects to the plasma replacement. Five patients were transferred to the maintenance plasmapheresis: three sessions were performed in total. One patient after the induction plasmapheresis sessions underwent only two procedures of maintenance plasmapheresis – the third maintenance plasmapheresis was denied due to the abdominal pain arising. The pain appeared three weeks later after the last (second) maintenance plasmapheresis. The appendicitis was diagnosed and the patient was operated. After the surgery plasma exchange was not performed more and the blood samples were not drawn later as the patient refused to participate in the study.

All patients before TPE did not have an *in vivo* MxA response to IFN- and all of them on the basis of MxA

Table 2. **MxA mRNA expression and MxA mRNA induction levels before and after induction and maintenance TPE in six MS patients.**

MxA mRNA expression cut-off of <0.586 and MxA mRNA induction cut-off of <3.84 were considered negative. Adequate biological responders were defined as patients with both expression and induction levels higher than cut-off. Patients with both expression and induction levels lower than cut-off – as biological non-responders, and patients with either an increase of expression or induction cut-off were called suboptimal responders.

Patient 1

	Before TPE	After centrifugal TPE	1 mth after centrifugal TPE	3 mth after centrifugal TPE
MxA mRNA expression	0.815	1.2311	0.76	0.39
MxA mRNA induction	0.98	5.55	1.87	1.4

Patient 2

	Before TPE	After induction TPE	1 mth after maintenance TPE	2 mth after maintenance TPE	3 mth after maintenance TPE	4 mth after 3 maintenance TPE sessions
MxA mRNA expression	1.59	4.94	5.01	4.19	2.36	2.16
MxA mRNA induction	1.43	4.64	5.37	3.64	1.77	1.69

Patient 3

	Before TPE	After induction TPE	1 mth after maintenance TPE	2 mth after maintenance TPE	3 mth after maintenance TPE	4 mth after 3 maintenance TPE sessions
MxA mRNA expression	2.85	3.29	6.1	3.69	2.77	1.82
MxA mRNA induction	1.69	7.38	1.93	2.9	1.77	1.54

Patient 4

	Before TPE	After induction TPE	1 mth after maintenance TPE	2 mth after maintenance TPE	3 mth after maintenance TPE	4 mth after maintenance TPE sessions
MxA mRNA expression	3.08	6.4	2.41	4.4	1.43	2.23
MxA mRNA induction	2.66	19.63	10.74	2.86	2.16	4.64

Patient 5

	Before TPE	After induction TPE	1 mth after maintenance TPE	2 mth after maintenance TPE
MxA mRNA expression	3.24	4.77	2.51	0.87
MxA mRNA induction	2.03	3.05	3.18	1.35

Patient 6

	Before TPE	After induction TPE	1 mth after maintenance TPE	2 mth after maintenance TPE	3 mth after maintenance TPE	4 mth after maintenance TPE sessions
MxA mRNA expression	1.83	2.16	1.3	1.12	0.93	1.48
MxA induction	3.37	2.19	4.53	4.22	2.32	3.41

MxA – myxovirus resistance protein A; mRNA – messenger RNA; TPE – therapeutic plasma exchange.

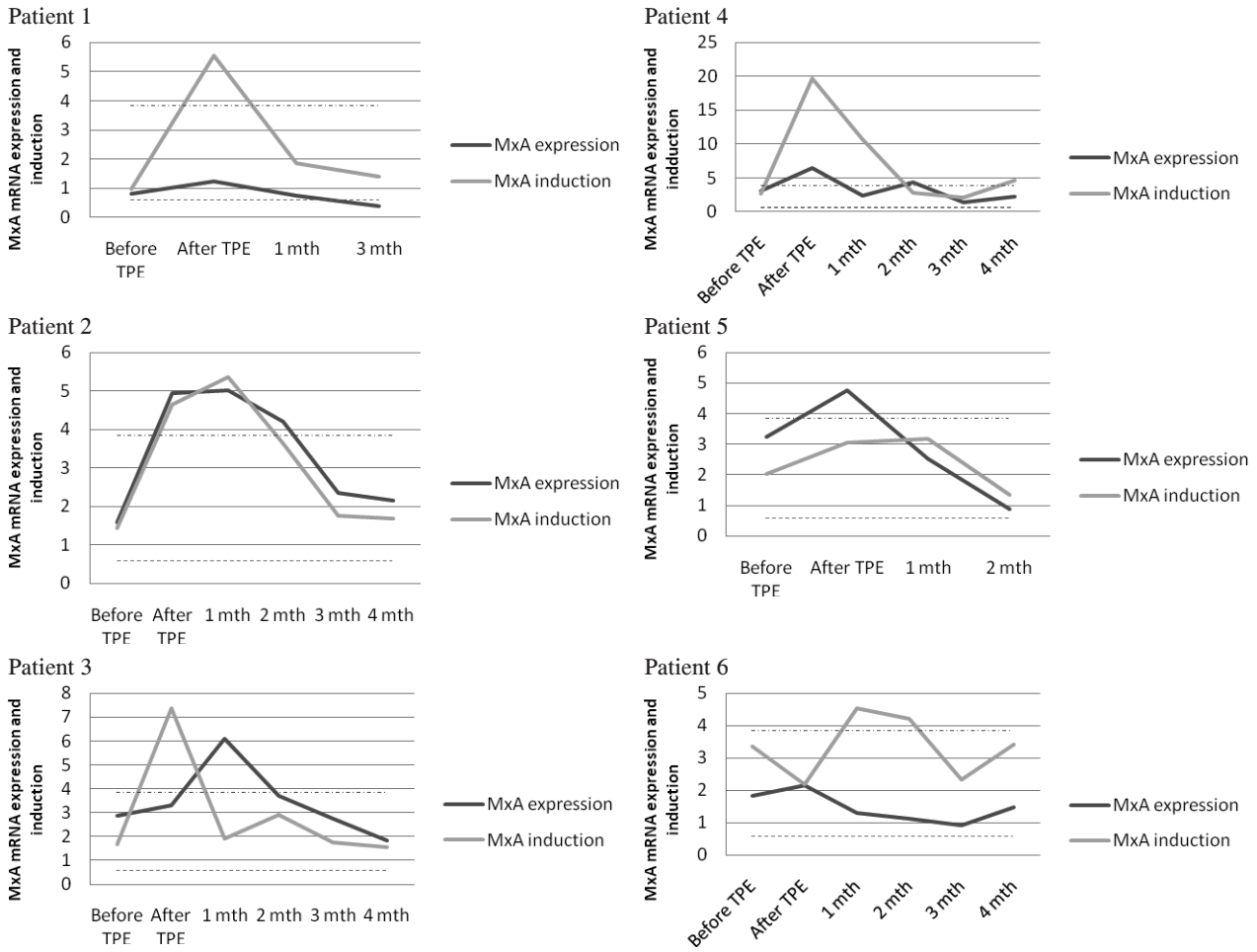

Figure. **MxA mRNA expression and MxA mRNA induction levels before and after four sessions of induction TPE and after maintenance TPE each subsequent month in six MS patients separately.**
MxA mRNA expression cut-off is shown as a dashed line, MxA mRNA induction cut-off – as a dot-dashed line.
MxA – myxovirus resistance protein A; mRNA – messenger RNA; TPE – therapeutic plasma exchange.

mRNA expression results were recognized "as poor biological responders". A sharp increase of MxA mRNA expression and induction was found in four patients after induction plasmapheresis (in one case after centrifugal plasmapheresis): the patients regained an *in vivo* MxA response to IFN- therapy and became "biological responders". In two patients an increase of MxA mRNA expression or induction was found but the values persisted below the lower cut-off and the patients remained as "poor biological responders". The effect of maintenance plasmapheresis was very transient: MxA mRNA expression values reverted to the baseline levels after one or two months (Table 2, Figure).

Safety and tolerability of the TPE. One of the six patients after the first incomplete plasma exchange session was switched to the centrifugal plasmapheresis (MxA mRNA expression results after centrifugal plasmapheresis were done as scheduled) due to the excessive itchy rashes on the trunk, neck and upper limbs (without hypotension), which persisted after the additional multiple dexamethasone and clemastine doses. One patient during the first and the second session of induction plasmapheresis had urticaria on the face and neck. The skin rash had regressed completely after the additional dose of dexamethasone and clemastine and the sessions were completed without any other adverse events. All other patients tolerated plasma exchange well when donor plasma was used for plasma replacement. In this trial serious adverse events were not observed during and after TPE sessions.

DISCUSSION AND CONCLUSIONS

Most RRMS patients treated with IFN- preparations do not develop persistent and high-titer NAbs that have been associated with reduced measures of radiographic and clinical efficacy. For those that do, however, NAbs represent a significant clinical problem, especially if other therapies have already been used, are not tolerated, or are not available. The available evidence for prevention and treatment of NAbs in IFN- treated RRMS patients still is scant and methodologically weak. Several studies proved that treatment with monthly cycles of high-dose methylprednisolone did not restore IFN- biologic response and did not

reduce NAbs levels in established NAB-positive MS patients, as well as cyclic methylprednisolone given together with IFN- from the beginning of the treatment [41, 43]. Even a combination of azathioprine and monthly methylprednisolone cycles has little or no effect on IFN- bioactivity in NAb-positive patients with MS [44].

As it is well known, TPE is effectively and widely used to remove serum proteins from the circulation, so we decided in the present study to make the induction and maintenance TPE in order to restore and then sustain the recovered bioavailability of IFN- . As far as we know, such attempts of reducing NAbs and/or restoring the bioavailability of IFN- has not been described previously. All the patients in this study were treated with IFN- for more than 18 months and did not have an *in vivo* MxA response to IFN- . We consider the *in vivo* response to IFN- to be a more accurate estimate of IFN- bioavailability in the individual patient, as *ex vivo* by CPE detected NAbs may have different and sometimes low affinities, and not always have neutralizing activity *in vivo*, and as the cut-off for NAbs positivity is set somewhat arbitrarily [42]. Moreover, several studies have shown that whereas high titers of NAbs invariably are associated with decreased IFN- bioactivity, low NAbs titers are correlated poorly with expression IFN- induced genes or gene products [30, 54, 55]. The present data has supported the hypothesis that induction TPE may promote the recovery of the *in vivo* biological response to IFN- , as two categories of patients after induction plasmapheresis were identified: first category participants (total – four patients) became "biological responders" and two patients persisted as "poor biological responders". However, we cannot rule out the possibility that the bioactivity of IFN- has not been restored in two patients as they were older (54 and 49 years old) and due to the long duration of treatment (6 and 5 years respectively). They were the oldest of all other patients and occured with the longest treatment duration: the mean age of all other patients was 41.8 years and the treatment duration was 2.75 years. Unfortunately, the effect of maintenance plasmapheresis was transient: the biological activity of IFN- has been maintained from one to two months on average. Therefore, this method of treatment cannot be applied for continuous diminishing of NAb titers and sustaining of IFN- bioavailability.

Of course, it is useful to keep in mind that the use of TPE in chronic diseases and conditions is more controversial than in acute self-limited diseases due to the phenomenon of rebound antibody production and because it does not address underlying pathology. Although TPE can rapidly reduce levels of serum autoantibodies, a feedback mechanism in chronic diseases, and maybe in this context, may lead to a rebound overproduction of the same antibodies.

In conclusion the results of the previous and the present study demonstrate that an optimal methodology to restore or improve the markers of IFN- bioactivity in abolished IFN- ability or to prevent the development of NAbs in IFN- treated RRMS patients is not yet available. Consequently, to tailor the best treatment for MS patients and to prevent the development of NAbs, the neurologist must carefully consider the results of clinical trials, disentangle the peculiar individual clinical and prognostic characteristics of each patient, susceptibility to NAbs and clinical effects of therapy.

Gauta: 2013 10 14
Priimta spaudai: 2013 10 28

References

1. Kieseier BC. The mechanism of action of interferon- in relapsing multiple sclerosis. CNS Drugs 2011; 25(6): 491–502.
2. Dhib-Jalbut S, Marks S. Interferon-beta mechanisms of action in multiple sclerosis. Neurology 2010; 74: 17–24.
3. Schellekens H. Bioequivalence and the immunogenicity of biopharmaceuticals. Nat Rev Drug Discov 2002; 1(6): 457–62.
4. Sorensen PS. Neutralizing antibodies against interferon-beta. Ther Adv Neurol Disord 2008; 1(2): 62–78.
5. Li DK, Paty DW. Magnetic resonance imaging results of the PRISMS trial: a randomized, double-blind, placebo-controlled study of interferon-beta1a in relapsing-remitting multiple sclerosis. Prevention of Relapses and Disability by Interferon-beta1a Subcutaneously in Multiple Sclerosis. Ann Neurol 1999; 46(2): 197–206.
6. PRISMS Study Group and the University of British Columbia MS/MRI Analysis Group. PRISMS-4: Long-term efficacy of interferon-beta-1a in relapsing MS. Neurology 2001; 56(12): 1628–36.
7. European Study Group on Interferon -1b in Secondary Progressive MS. Placebo-controlled multicentre randomised trial of interferon beta-1b in treatment of secondary progressive multiple sclerosis. European Study Group on interferon beta-1b in secondary progressive MS. Lancet 1998; 352(9139): 1491–7.
8. Polman C, Kappos L, White R, et al; European Study Group in Interferon Beta-1b in Secondary Progressive MS. Neutralizing antibodies during treatment of secondary progressive MS with interferon beta-1b. Neurology 2003; 60(1): 37–43.
9. Sorensen PS, Ross C, Clemmesen KM, Bendtzen K, et al; Danish Multiple Sclerosis Study Group. Clinical importance of neutralising antibodies against interferon beta in patients with relapsing-remitting multiple sclerosis. Lancet 2003; 362(9391): 1184–91.
10. Goodin DS, Hartung HP, O'Connor P, et al. Neutralizing antibodies to interferon beta-1b multiple sclerosis: a clinico-radiographic paradox in the BEYOND trial. Mult Scler 2012; 18(2): 181–95.
11. Francis GS, Rice GP, Alsop JC; PRISMS Study Group. Interferon beta-1a in MS: results following development of neutralizing antibodies in PRISMS. Neurology 2005; 65(1): 48–55.
12. Minagara A, Murray TJ; PROOF Study Investigators. Efficacy and tolerability of intramuscular interferon beta-1a compared with subcutaneous interferon beta-1a in relapsing MS: results from PROOF. Curr Med Res Opin 2008; 24(4): 1049–55.
13. Secondary Progressive Efficacy Clinical Trial of Recombinant Interferon-Beta-1a in MS (SPECTRIMS) Study Group. Randomized controlled trial of interferon- beta-1a in second-

ary progressive MS: Clinical results. Neurology 2001; 56(11): 1496–504.
14. Panitch H, Miller A, Paty D, et al; North American Study Group on Interferon beta-1b in Secondary Progressive MS. Interferon beta-1b in secondary progressive MS: results from a 3-year controlled study. Neurology 2004; 63(10): 1788–95.
15. Kappos L, Clanet M, Sandberg-Wollheim M, et al; European Interferon Beta-1a IM Dose-Comparison Study Investigators. Neutralizing antibodies and efficacy of interferon beta-1a: a 4-year controlled study. Neurology 2005; 65(1): 40–7.
16. Serana F, Imberti L, Amato M, et al. The study of IFN bioactivity loss by MxA mRNA quantification patients allows the prediction of disability progression in multiple sclerosis patients. Neurology 2013; 80(Meeting Abstracts 1): P04.140.
17. Ross C, Clemmesen KM, Svenson M, et al. Immunogenicity of interferon-beta in multiple sclerosis patients: influence of preparation, dosage, dose frequency, and route of administration. Danish Multiple Sclerosis Study Group. Ann Neurol 2000; 48(5): 706–12.
18. Sominanda A, Rot U, Suoniemi M, et al. Interferon beta preparations for the treatment of multiple sclerosis patients differ in neutralizing antibody seroprevalence and immunogenicity. Mult Scler 2007; 13(2): 208–14.
19. Gneiss C, Tripp P, Reichartseder F, et al. Differing immunogenic potentials of interferon beta preparations in multiple sclerosis patients. Mult Scler 2006; 12(6): 731–7.
20. Prince HE, Lapé-Nixon M, Audette C, et al. Identification of interferon-beta antibodies in a reference laboratory setting: findings for 1144 consecutive sera. J Neuroimmunol 2007; 190(1–2): 165–9.
21. Perini P, Calabrese M, Biasi G, et al. The clinical impact of interferon beta antibodies in relapsing-remitting MS. J Neurol 2004; 251(3): 305–9.
22. Petkau AJ, White RA, Ebers GC, et al; IFNB Multiple Sclerosis Study Group. Longitudinal analyses of the effects of neutralizing antibodies on interferon beta-1b in relapsing-remitting multiple sclerosis. Mult Scler 2004; 10(2): 126–38.
23. Herndon RM, Rudick RA, Munschauer FE 3rd, et al. Eight-year immunogenicity and safety of interferon beta-1a-Avonex treatment in patients with multiple sclerosis. Mult Scler 2005; 11(4): 409–19.
24. Petersen B, Bendtzen K, Koch-Henriksen N, et al; Danish Multiple Sclerosis Group. Persistence of neutralizing antibodies after discontinuation of IFNbeta therapy in patients with relapsing-remitting multiple sclerosis. Mult Scler 2006; 12(3): 247–52.
25. Van der Voort LF, Gilli F, Bertolotto A, et al. Clinical effect of neutralizing antibodies to interferon beta that persist long after cessation of therapy for multiple sclerosis. Arch Neurol 2010; 67(4): 402–7.
26. Farrell RA, Marta M, Gaeguta AJ, et al. Development of resistance to biologic therapies with reference to IFN- . Rheumatology (Oxford) 2012; 51(4): 590–9.
27. Creeke PI, Farrell RA. Clinical testing for neutralizing antibodies to interferon- in multiple sclerosis. Ther Adv Neurol Disord 2013; 6(1): 3–17.
28. Bertolotto A, Sala A, Caldano M, et al. Development and validation of a real time PCR-based bioassay for quantification of neutralizing antibodies against human interferon-beta. J Immunol Methods 2007; 321: 19–31.
29. Hesse D, Sellebjerg F, Sorensen PS. Absence of MxA induction by interferon beta in patients with MS reflects complete loss of bioactivity. Neurology 2009; 73: 372–7.
30. Bertolotto A, Gilli F, Sala A, et al. Persistent neutralizing antibodies abolish the interferon beta bioavailability in MS patients. Neurology 2003; 60: 634–9.
31. Malucchi S, Gilli F, Caldano M, et al. One-year evaluation of factors affecting the biological activity of interferon beta in multiple sclerosis patients. J Neurol 2011; 258: 895–903.
32. Farrell R, Kapoor R, Leary S, et al. Neutralizing anti-interferon beta antibodies are associated with reduced side effects and delayed impact on efficacy of Interferon-beta. Mult Scler 2008; 14: 212–8.
33. Lam R, Farrell R, Aziz T, et al. Validating parameters of a luciferase reporter gene assay to measure neutralizing antibodies to IFNbeta in multiple sclerosis patients. J Immunol Methods 2008; 336: 113–8.
34. Pachner AR, Warth JD, Pace A, et al; INSIGHT investigators. Effect of neutralizing antibodies on biomarker responses to interferon beta: the INSIGHT study. Neurology 2009; 73(18): 1493–500.
35. Santos R, Weinstock-Guttman B, Tamaño-Blanco M, et al. Dynamics of interferon-beta modulated mRNA biomarkers in multiple sclerosis patients with anti-interferon-beta neutralizing antibodies. J Neuroimmunol 2006; 176(1–2): 125–33.
36. Pachner AR, Dail D, Pak E, et al. The importance of measuring IFNbeta bioactivity: monitoring in MS patients and the effect of anti-IFNbeta antibodies. J Neuroimmunol 2005; 166(1–2): 180–8.
37. Van der Voort LF, Kok A, Visser A, et al. Interferon-beta bioactivity measurement in multiple sclerosis: feasibility for routine clinical practice. Mult Scler 2009; 15(2): 212–8.
38. Sominanda A, Hillert J, Fogdell-Hahn A. In vivo bioactivity of interferon-beta in multiple sclerosis patients with neutralising antibodies is titre-dependent. J Neurol Neurosurg Psychiatry 2008; 79(1): 57–62.
39. Durelli L, Ricci A, Bergui P, et al. High-dose (375 mcg) interferon beta-1b treatment of multiple sclerosis and neutralizing antibodies. Long-term follow-up. J Neurol 2006; 253(suppl. 2): 100.
40. Pozzilli C, Antonini G, Bagnato F, et al. Monthly corticosteroids decrease neutralizing antibodies to IFNbeta1 b: a randomized trial in multiple sclerosis. J Neurol 2002; 249(1): 50–6.
41. Zarkou S, Carter JL, Wellik KE, et al. Are corticosteroids efficacious for preventing or treating neutralizing antibodies in multiple sclerosis patients treated with beta-interferons? A critically appraised topic. Neurologist 2010; 16(3): 212–4.
42. Hesse D, Frederiksen JL, Koch-Henriksen N, et al. Methylprednisolone does not restore biological response in multiple sclerosis patients with neutralizing antibodies against interferon- . Eur J Neurol 2009; 16(1): 43–7.
43. Cohen JA, Imrey PB, Calabresi PA, et al. Results of the Avonex Combination Trial (ACT) in relapsing-remitting MS. Neurology 2009; 72(6): 535–41.
44. Ravnborg M, Bendtzen K, Christensen O, et al. Treatment with azathioprine and cyclic methylprednisolone has little or no effect on bioactivity in anti-interferon beta antibody-positive patients with multiple sclerosis. Mult Scler 2009; 15(3): 323–8.
45. Winters JL. Plasma exchange: concepts, mechanisms, and an overview of the American Society for Apheresis guidelines. Hematology Am Soc Hematol Educ Program 2012; 2012: 7–12.
46. Stegmayr B, Ptak J, Wikström B. World apheresis registry report. Transfus Apher Sci 2007; 36(1): 13–6.

47. Norda R, Stegmayr BG; Swedish Apheresis Group. Therapeutic apheresis in Sweden: update of epidemiology and adverse events. Transfus Apher Sci 2003; 29(2): 159–66.
48. Korach JM, Petitpas D, Paris B, et al; French Registry Study Group. Plasma exchange in France: Epidemiology 2001. Transfus Apher Sci 2003; 29(2): 153–7.
49. Rock G, Clark B, Sutton D; CAG; CAAN. The Canadian apheresis registry. Transfus Apher Sci 2003; 29(2): 167–77.
50. Mokrzycki MH, Balogun RA. Therapeutic apheresis: a review of complications and recommendations for prevention and management. J Clin Apher 2011; 26(5): 243–8.
51. Rudick RA, Goodkin DE. Multiple sclerosis therapeutics. London: Martin Dunitz, 1999.
52. Pachner AR, Narayan K, Pak E. Multiplex analysis of expression of three IFNbeta-induced genes in antibody-positive MS patients. Neurology 2006; 66(3): 444–6.
53. Gilli F, Marnetto F, Caldano M, et al. Biological responsiveness to first injections of interferon-beta in patients with multiple sclerosis. J Neuroimmunol 2005; 158(1–2): 195–203.
54. Pachner A, Narayan K, Price N, et al. MxA gene expression analysis as an interferon-beta bioactivity measurement in patients with multiple sclerosis and the identification of antibody-mediated decreased bioactivity. Mol Diagn 2003; 7(1): 17–25.
55. Bertolotto A, Gilli F, Sala A, et al. Evaluation of bioavailability of three types of IFNbeta in multiple sclerosis patients by a new quantitative-competitive-PCR method for MxA quantification. J Immunol Methods 2001; 256(1–2): 141–52.

N. Giedraitiene, R. Kizlaitiene, V. Budrys, G. Kaubrys, L. Griskevicius, V. Valceckiene, M. Stoskus, A. Griskevicius, J. Audzijoniene

PAKAITINIŲ PLAZMAFEREZIŲ ĮTAKA INTERFERONO BETA BIOLOGINIAM AKTYVUMUI. PILOTINĖ STUDIJA

Santrauka

Neutralizuojantys antikūnai (NAk) prieš interferoną beta (IFN-) sumažina arba visiškai panaikina IFN- biologinį aktyvumą. Aukšti NAk titrai turi įtakos išsėtinės sklerozės (IS) paūmėjimų skaičiui ir neigiamą poveikį ligos MRT aktyvumui. Pakaitinė plazmaferezė yra gydymo metodas, kuris pašalina patogeninius antikūnus, imuninius kompleksus ir kitas medžiagas, kurios, cirkuliuodamos organizme, tampa ligos priežastimi. Šio tyrimo tikslas buvo įvertinti pacientų, sergančių RRIS ir gydomų IFN- , indukcinių pakaitinių plazmaferezių įtaką IFN- gebėjimui indukuoti MxA baltymo geno mRNR atstatymą, taip pat įvertinti palaikomųjų plazmaferezių įtaką IFN- biologiniam aktyvumui išlaikyti.

Metodai. Įtraukimo kriterijus atitikusiems pacientams buvo atliktos keturios indukcinės pakaitinės plazmaferezės IFN- biologiniam aktyvumui atstatyti. Po indukcinių plazmaferezių kurso pacientai pervesti prie palaikomųjų plazmaferezių. Interferono beta biologiniam aktyvumui nustatyti taikytas MxA mRNR indukcijos tyrimas.

Rezultatai. Buvo įtraukti šeši pacientai, sergantys RRIS ir turintys dalinį IFN- biologinį aktyvumą skriningo metu. Atlikus keturias indukcines plazmaferezes, keturiems pacientams atstatytas IFN- biologinis aktyvumas. Dviem pacientams po indukcinių plazmaferezių kurso stebėtas MxA mRNR ekspresijos padidėjimas, tačiau rodikliai išliko žemiau slenkstinės normos ribos, taip pat išliko ir dalinis IFN- biologinis aktyvumas. Atliekant palaikomąsias plazmaferezes, IFN- biologinio aktyvumo rodikliai grįžo į pradinį lygį po vieno dviejų mėnesių.

Išvados. Pakaitinės plazmaferezės kai kuriems pacientams gali atstatyti IFN- biologinį aktyvumą, tačiau palaikomųjų plazmaferezių įtaka IFN- biologinio aktyvumo rodikliams yra trumpalaikė.

Raktažodžiai: Interferonas beta, neutralizuojantieji antikūnai, interferono- biologinis aktyvumas, MxA mRNR ekspresija, pakaitinė plazmaferezė.