Study Identification

Unique Protocol ID: IFCI-25/05/2015
Brief Title: Intranasal Inhalations of Bioactive Factors Produced by M2 Macrophages in Patients With Organic Brain Syndrome
Official Title: Safety/Efficacy of Intranasally-Administered Bioactive Factors Produced by Autologous M2 Macrophages in Patients With Organic Brain Syndrome

The protocol was approved by the Ethics Committee of the Institute of Fundamental and Clinical Immunology (approval number: no 92, 11/10/2015)

Study Protocol **October 11, 2015**

Scientific background

Studies over the last two decades have demonstrated that macrophages could cause not only neurodestructive but also neuroreparative effects, with macrophage activation being an essential prerequisite for the functional restoration of central nervous system (CNS) following damage [1, 2, 3, 4]. This dual impact is related to the heterogeneity of macrophages [5]. Classically activated M1-type macrophages with pro-inflammatory phenotype possess neurotoxic properties, while M2-type alternatively activated macrophages with anti-inflammatory properties mediate neuroprotection, activate neuro- and angio-genesis, and play an essential role in neuronal plasticity and axon remodelling [4, 6, 7].

Macrophages within CNS comprise microglia (the dominant macrophage population) and monocyte-derived CNS-infiltrating macrophages. Upon recognition of danger molecules, short-term or moderate signal directs microglia toward M2 regenerative phenotype, while intensive acute or chronic activation induces M1 neurotoxic phenotype [8]. In last case, neuroreparative deficiency is likely to be compensated by recruiting peripheral blood monocytes and their M2 polarisation [9, 10]. In accordance with this notion, therapeutic approaches targeting macrophage polarisation towards M2 phenotype has been considered as a novel strategy for stimulating neuroreparative processes [11, 12].

We have previously developed an original protocol for obtaining M2-like macrophages based on macrophage interaction with apoptotic cells [13]. As compared to M1, M2 macrophages generated were characterised by low antigen-presenting and pro-inflammatory activity, while possessing more pronounced regeneration potential due to high production of a variety of growth and neurotrophic factors [13, 14]. Pilot studies of M2 intrathecal administrations in ischemic stroke [15] and severe cerebral palsy [16, 17] demonstrated the safety and amelioration of motor and cognitive functions. However, the invasiveness of intrathecal injection and the possibility of M2→M1 repolarization in pathological microenvironment were serious factors limiting clinical translation of this treatment. In this connection, the utilization of M2-derived bioactive factors (M2-BFs) instead of cells and intranasal administration of M2-BFs that allows for rapid delivery of various substances (including neurotrophic factors) in brain tissues [18] via olfactorial and trigeminal route bypassing blood-brain barrier appears to be a promising approach [19, 20, 21]. A possibility for cytokines and hormones to be delivered into brain tissue upon intranasal route of delivery has been demonstrated in rodent and primate experimental models [22] as well as in human being [23, 24].

Since conditioned M2 macrophage culture medium contains a wide spectrum of neurotrophic, pro-angiogenic and immunoregulatory cytokines, we have designed an innovative proof-of-concept trial designed to provide data as to whether the treatment/rehabilitation efficacy and functional outcome of patients with organic brain syndrome are improved with intranasal inhalations of bioactive factors, produced by autologous M2 macrophages (auto-M2-BFs).

The aim of this study was to assess safety and clinical effectiveness of intranasal administration of bioactive factors, produced by autologous M2 macrophages in patients with organic brain syndrome.

Study Design
Study Type: Interventional
Primary Purpose: Treatment
Study Phase: Phase 1/Phase 2
Intervention Model: Single Group Assignment
Number of Arms: 1
Masking: No masking
Enrollment: 30

Criteria:
Inclusion Criteria:

- Adults: age 18 - 80
- Persistent neurological deficits (cognitive, mental, motor, vestibular/ataxic disorders as a result of trauma, cardiovascular, neurodegenerative and others cerebral injuries), confirmed clinically and by CT or MRI
- A written informed consent of the patient or close relatives

Exclusion Criteria:

- Psychiatric disorders
- Seizures
- Severe dementia
- Hepatic or renal dysfunctions
- Hemodynamic or respiratory instability
- HIV or uncontrolled bacterial, fungal, or viral infections
- Pregnancy
- Malignancy
- Intolerance to gentamicin and / or multiple drug allergies
- Participation in other clinical trials

Outcome Measures
Primary Outcome Measure:
1. The Number of Patients With Severe Adverse Events and Adverse Reactions
Time Frame: up to 6 months after treatment
Measure Description: Occurrence of severe adverse events and adverse reactions (allergic, toxic, inflammatory reactions; neurological deterioration, convulsive syndrome)

Secondary Outcome Measure:

2. Change in Subjective Assessment of Clinical Symptoms (SACS)

Time Frame: Baseline and 6 months after treatment

Measure Description: Subjective Assessment of Clinical Symptoms (SACS) is a 5-point rating scale with standardized criteria (0 – no; 1 – mild; 2 – moderate; 3 – severe; 4 – intensive) subjective assessment of the severity of fifteen clinical symptoms most characteristic of neurological disorders (headache, dizziness, gait disturbance, speech, visual impairment, tremor et al). Minimum SACS "total" score is 0, and maximum SACS "total" score is 60. Neurological improvements are assessed by SACS "total" score as > 6 points' reduction from baseline.

3. Change in Hospital Anxiety and Depression Scale (HADS)

Time Frame: Baseline and 6 months after treatment

Measure Description: Hospital Anxiety and Depression Scale (HADS) is used to diagnose anxiety/depression symptoms (absence – 0~7 points; subclinical form – 8~10 points; clinical form – 11 points or more). Minimum HADS "total" score (anxiety +depression subscale) is 0, and maximum HADS "total" score is 42. Improvements in patients with anxiety/depression symptoms are assessed by HADS "total" score as > 4 points reduction from baseline.

4. Change in Functional Mobility Assessment (FMA) Scale

Time Frame: Baseline and 6 months after treatment

Measure Description: Functional Mobility Assessment (FMA) is cale is designed to evaluate parameters characterizing stability (0~24 points) and gait (0~16 points). The maximum FMA "total" score on stability and gait subscales is 39-40 and corresponds to the norm, minimum FMA "total" score is 0 and corresponds to the gross impairment. The degree of impairment of "total" score is divided into significant (0~20 points), moderate (21~33 points), and light (34~38 points), whereas 39~40 points indicate no impairments. Improved mobility is assessed as FMA "total" score enhancement > 4 points from baseline.

5. Change in Montreal Cognitive Assessment (MoCA)

Time Frame: Baseline and 6 months after treatment

Measure Description: Montreal Cognitive Assessment (MoCa) is used to assess cognitive functions. The maximum MoCa "total" score is 26-30 points and corresponds to the norm, 19-25 points - mild cognitive disorder; 11-21 points - dementia. Improvements in patients with cognitive disorder are assessed as MoCA "total" score increase > 3 points from baseline.

Methods

M2-BFs preparation

M2 macrophages are generated from adherent mononuclear cells (MNCs), as described previously [13, 14]. Briefly, MNCs are separated standardly from heparinized venous blood (150-200 ml) of a patient. The plastic-adherent MNCs are cultured in RPMI-1640 medium containing 0.05 mM 2-mercapethanol, 2 mM sodium pyruvate, 0.3 mg / ml L-glutamine, 1% solution of essential amino acids, 100 μg/ml gentamicin, 2% autoplasma, and recombinant human GM-CSF (rhGM-CSF, 50 ng/ml, R&D Systems) for 7 days. Conditioned medium of M2 macrophages is subjected to sterilizing filtration and collected in sterile vials (2.0 ml/vial), which are labeled as M2-BFs and stored at -20° C.

Intranasal M2-BFs administration

The conditioned medium of patient M2 macrophages (2.0 ml) is thawed at room temperature and used as a fine aerosol intranasally using a compressor inhaler (nebulizer). Patient will receive their first doses (n=2-3) of M2-BFs in clinic under the supervision of a physician and wait 2 hrs to determine any short-time adverse effects of inhaled dose. The subsequent course of intranasal inhalations (once a day up to 30 days) performed as outpatient treatment.

Statistical Analysis

Data will be analyzed using Statistica 6.0 software for Windows (StatSoft Inc. USA). The results of statistical analysis will be presented as median (Me) and interquartile range (IQR; LQ-UQ). To check the normality of the distribution of data, the Kolmogorov-Smirnov test and the W Shapiro-Wilk test will be used. To assess the significance of differences, Fisher's exact test (for discrete variables) and the non-parametric Mann-Whitney test (for continuous variables) will be used. Spearman's rank correlation coefficient (RS) will be used to measure the statistical dependence between two continuous variables. Differences with p less than 0.05 will be considered statistically significant.

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