Metabolic Syndrome and Neurotrophins: Effects of Metformin and Non-Steroidal Antiinflammatory Drug Treatment

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Abstract

Objective: Metabolic syndrome (MS) presents with central obesity, impaired glucose metabolism, dyslipidemia and hypertension. Our aim was to examine the effect of metformin treatment either alone or in combination with non-steroidal anti-inflammatory drugs (NSAID) on plasma levels of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in patients with early stage MS (MS-es) and generalized MS (MS-ge).

Materials and Methods: The study compared 35 female patients with MS-es (mean age of 43.39±1.54 years) and 40 patients with MS-ge (mean age of 45.69±2.18 years) to 10 age-matched controls each. Patients with MS-es were administered 850 mg metformin twice daily. The patients with MS-ge were divided into two groups of 20 patients per group. One group received metformin alone, while the other group received metformin in combination with 500 mg aspirin and 150 mg Diclac daily. Plasma NGF and BDNF levels were measured by ELISA. Statistical data analysis was performed using ANOVA.

Results: Plasma NGF and BDNF levels were significantly higher in MS-es patients and lower in MS-ge patients than in controls. NGF levels were decreased in both groups after treatment with metformin. NGF levels were significantly higher in MS-ge patients on combined therapy than in those on metformin only.

Conclusion: The combination of metformin and NSAID treatment is more effective than metformin alone on NGF and BDNF production as well as on metabolism-related anthropometric and laboratory features. This represents a pathogenetic therapeutic mechanism in MS due to its strong anti-inflammatory effect and improves MS-related symptoms.

Key Words: Brain-derived neurotrophic factor, Early stage metabolic syndrome, Generalized metabolic syndrome, Inflammation, Nerve growth factor

Özet

Amaç: Metabolik sendrom (MS), santral obezite, bozulmuş glukoz metabolizması, dislipidemi ve hipertansiyon ile kendini gösterir. Hedefimiz, tek başına metformin tedavisinin ve erken evre MS’li hastalarda (MS-es) ve generalize MS (MS-ge) sinir büyüme faktörü (NGF) ve beyin kaynaklı nörotrofik faktör (BDNF) plazma düzeylerine üzerine non-steroid antienflamatuar ilaçlar (NSAID) ile birlikte etkisini araştırmaktır.

Gereç ve Yöntem: Çalışma, MS-es’li 35 kadın hasta (yaş ortalaması 43.39±1.54 yıl) ve 40 MS-ge’li kadın hasta (yaş ortalaması 45.69±2.18 yıl) ve her biri ile yaş olarak eşleştirilmiş 10 hastadan oluşan kontrol grubu ile yapılmıştır. MS-es ile hastalar günde iki kez 850 mg dozda metformin verilmiştir. MS-ge ile hastalar, her biri 20 hasta iki grubu ayrıılır. Bunlar, 150 mg günlük doz 500 mg ve Diclac (Diklofenak Na) bir doz aspirin ile ya tek başına veya kombin olarak metformin almıştır. Plazma NGF ve BDNF düzeyleri ELISA yöntemi ile ölçüldü. İstatistik veri işleme, ANOVA yöntemi ile yapıldı.

Bulgular: Plazma NGF ve BDNF MS-es hastalarında önemli ölçüde daha yüksek ve MS-ge olanlar kontrollere göre daha düşüktü. NGF düzeyleri metformin ile tedavi sonrasında her iki grupta da azalmıştır. NGF seviyeleri sadece bu on metformin kombin tedavi MS-ge hastaarda daha anlamlı olarak yüksek bulunmuştur.

Sonuç: Metformin ve NSAID kombinasyonu NGF ve BDNF üretimi yanı sıra, metabolizma ile ilgili antropometrik ve laboratuar özellikler üzerine tek başına metforminden daha iyi bir etki gösterir. Bu tedavi, güçlü antiinflamatuar etkisi sebebiyle MS’de bir patogenezeyi yönelik tedaviyi yansıtır ve MS-ge belirtilerini iyileştirir.

Anahtar Kelimeler: Beyin kaynaklı nörotrofik faktör, Erken evre metabolik sendrom, Generalize metabolik sendrom, İnflamasyon, Nöronal büyüme faktörü
**Introduction**

Metabolic syndrome (MS) is characterized by central obesity, impaired glucose metabolism, dyslipidemia and hypertension [1], insulin resistance [2] and high-sensitivity C-reactive protein (CRP) [3, 4]. MS is a well-recognized and common condition of the modern lifestyle and is one of the most complex and heterogeneous of the metabolic diseases [5, 6]. New pathophysiological data imply that MS is a real disease and its prevalence is increasing worldwide [7, 8]. Proper identification of MS among diabetes mellitus (DM) patients is critical so that MS patients are properly treated using an integrated approach that reduces high costs and MS associated disabilities [9]. MS presents with DM type 2 (DMT2) features, several cardiometabolic risk factors and increases in cardiovascular mortality [10].

Neurotrophins are signaling proteins discovered because of their prosurvival role in neuronal cells, and they mediate neurotrophic, immunotrophic and metabotropic effects [11]. Neurotrophins are mainly produced in adipose tissue, salivary glands, and the hypothalamus by endocrine and immune cells, adipocytes, endothelial cells and keratinocytes [12-14]. Neurotrophins are considered key factors in MS pathogenesis, as any change in their plasma levels induces neuroendocrine-immune disturbances [15-17].

Metformin (Mf) (Glucophage, Glumetza, Fortamet, Riomet) is a glucose-sensitizing drug that increases the sensitivity of various tissues, such as muscle, liver and fat, to insulin uptake and activity [18]. No data are available about either the action of Mf or the influence of non-steroidal anti-inflammatory drugs (NSAIDs) on circulating levels of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) [19, 20].

The aim of this study was to examine the effect of Mf treatment alone and in combination with NSAIDs on plasma NGF and BDNF levels.

**Materials and Methods**

The study examined 35 female patients with early stage MS (MS-es) with a mean age of 43.39±1.54 years and 40 patients with generalized MS (MS-ge) with the mean age of 45.69±2.18 years. The patients with MS-es presented with 3 criteria of NCEP ATP III while those with MS-ge presented with >3 criteria. A total of twenty age-matched healthy, non-treated females (10 with a mean age of 43.75±2.53 years were compared to MS-es patients and 10 with a mean age of 42.50±2.75 years were compared to MS-ge patients) selected by these criteria served as controls. Familial obesity was excluded in the whole sample. Chronic infection confirmed by CRP expression was reported for all the participants in the study. Body mass index (BMI) and waist circumference were measured prior to and after treatment. Metabolic inflammatory variables such as serum levels of glucose, cholesterol and triglycerides were estimated by means of routine enzymatic methods. CRP was assessed using an Olympus apparatus and by the immunoturbidimetric method. Plasma NGF and BDNF levels were determined by ELISA in the Institute of Neurobiology and Molecular Medicine (Rome, Italy) both before drug treatment and after 5 months of treatment. The patients with MS-es were given 850 mg Mf twice daily for 5 months. The patients with Mf-ge were divided into two groups: one given 850 mg Mf alone twice daily for 5 months (n=20) and the other given Mf in combination with two NSAIDs, 500 mg aspirin for 15 days followed by 150 mg Diclac (diclofenac sodium) daily (n=20) for the next 15 days. Alternative anti-inflammatory therapy diminished the side effects of both drugs and improved patient's tolerance during the 5-month period.

Statistical data analysis was performed using variation analysis and analysis of variance (ANOVA); p values less than 0.05 were considered statistically significant. All of the measurements were taken after a written consent form was signed by the patient in compliance with the Helsinki convention.

**Results**

**NGF plasma levels**

Prior to treatment, statistically higher NGF levels (86.5 pg/mL±48.96 pg/mL) were observed in MS-es patients in comparison to control patients (36.3 pg/mL±18.2 pg/mL (Table 1 and Figure 1). After 5 months of Mf therapy, NGF levels were significantly reduced to 28.9 pg/mL±16.9 pg/mL compared to control levels (Table 1, Table 2 and Figure 1). Prior to treatment, statistically lower NGF levels (21.4 pg/mL±16.8 pg/mL) were established in MS-ge patients compared to control patients (36.3±18.2 pg/mL (Table 3 and Figure 1). After 5 months of Mf therapy, these levels were significantly reduced to 15.7 pg/mL±9.9 pg/mL when compared to pretherapy levels. Combined treatment of Mf with NSAIDs resulted in a significant increase in NGF levels to 36.1 pg/mL±24.9 pg/mL when compared to Mf only treated patients.

**Table 1. Neurotrophin plasma levels in MS before treatment with Mf**

<table>
<thead>
<tr>
<th>Neurotrophins (in pg/mL)</th>
<th>Healthy controls (n=10)</th>
<th>MS-es (n=35)</th>
<th>MS-ge (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGF</td>
<td>36.3±18.2</td>
<td>86.50±48.96*</td>
<td>21.47±16.8*</td>
</tr>
<tr>
<td>BDNF</td>
<td>2790.2±880.2</td>
<td>2880.99±807.3</td>
<td>2721.11±878.2</td>
</tr>
</tbody>
</table>

*p<0.05, †p<0.01
BDNF plasma levels

There were statistically higher BDNF levels (2880.99 pg/mL±807.35 pg/mL) in MS-es patients prior to treatment in comparison to controls (2790.2 pg/mL±880.2 pg/mL) (Table 1). There were statistically significantly lower BDNF plasma levels (2721.1±878.2 pg/mL) in MS-ge patients in comparison to controls (2790.2 pg/mL±880.2 pg/mL) (Table 1). However, there were no significant alterations in BDNF plasma levels in patients treated with Mf alone or in combination with NSAIDs (Table 3).

Metabolic parameters

There was a significant improvement in CRP, NGF, BMI and waist circumference in MS-es patients on Mf therapy (Table 2). BMI significantly decreased while a high CRP level persisted in MS-ge patients on Mf therapy only (Table 3). CRP and waist circumference significantly decreased while BMI decreased only slightly in MS-ge patients on both Mf and NSAID treatment (Table 3).

Discussion

Recently, large meta-analyses have examined the increased risk of cardiovascular events in people with MS [18-22]. These studies showed that MS was associated with a 2-fold increase in cardiovascular outcomes and a 1.5-fold increase in all-cause mortality rates while the cardiovascular risk remained high in patients with MS but without DM. Our results in this study confirm our previous data that neurotrophin levels increase in MS-es and decrease in MS-ge compared with control levels [16, 17, 23]. BDNF levels are elevated in individuals with high numbers of risk factors for MS [24].

Mf treatment improves carbohydrate and lipid metabolism in patients with MS-es. Mf diminishes visceral adipose tissue and exerts an anti-inflammatory effect that is likely due to the reduction of neurotrophin levels down to an almost normal level.

Because neurotrophins are growth factors, it seems that their elevation in MS-es is a risk factor for tumorigenesis. Mf possesses an anti-neoplastic activity [25]. Thus, the reduction in neurotrophins during MS-es treatment with Mf is a possible mechanism for utilization of the anti-neoplastic activity of this medication.

We established that Mf treatment during MS-ge statistically decreases neurotrophin levels. This reduction decreases carbohydrate and lipid metabolism as both BMI and visceral adipose tissue increase but CRP remains elevated potentially resulting in cardiometabolic complications. Thus, it can be postulated that, generally, the inflammatory conditions that occur in MS-ge cannot be controlled by Mf only. Mf is a widely used anti-diabetic drug. In addition to its classical effects on glucose and lipid metabolism, it inhibits low-grade inflammation and improves BMI in DM patients with MS [26, 27].

Recent studies reveal that NGF exerts various non-neuronal effects including stimulation of insulin secretion by pancreatic β-cells, and these cells synthesize and release NGF [28]. Furthermore, NGF and BDNF are implicated in the pathogenesis of cardiometabolic diseases including MS [16, 29, 30]. BDNF and its high affinity tyrosine kinase receptor trkB contribute to
food intake and body weight control, as trkB signaling directly modulates appetite and metabolism [14]. The trkB agonists mediate anorexic and weight-reducing effects and emerge as a potential therapy for MS [21]. Balancing the plasma levels of neurotrophins could favorably aid in managing MS.

Neurotrophins possess an anorexigenic effect and regulate food intake, energy usage and quantity of adipose tissue through central mechanisms and the ventromedial hypothalamus [24, 31-33]. According to some authors, neurotrophins are significantly decreased in MS patients [34].

DM2 is associated with chronic mild inflammation [35, 36]. A pro-inflammatory state in adipose tissue can lead to local insulin resistance and DMT2 [37-39]. Measurement of serum inflammatory parameters in patients with MS may be beneficial in detection and management of cardiovascular events and in the assessment of therapeutic efficacy [40]. The presence of chronic mild inflammation in MS suggests potential benefits of anti-inflammatory treatment [15, 41].

Recently, increasing attention is being paid to the anti-inflammatory treatment of insulin resistance and MS [15, 34, 41]. NSAIDs suppress most effects of the pro-inflammatory cytokines on the target tissues. NSAIDs are powerful inhibitors of prostaglandin production in the brain. Aspirin has been suggested for the treatment of MS [42].

Cardiovascular risk reduction in individuals with MS should include control of: i) obesity, unhealthy diet and lack of physical activity; ii) atherogenic dyslipidemia, hypertension, dysglycemia and prothrombotic state, and iii) insulin resistance [18, 43].

We conclude that the combination of Mf and NSAIDs is more effective than Mf alone with respect to NGF and BDNF production and metabolism-related anthropometric and laboratory features. Mf/NSAID combination therapy represents a pathogenetic therapy for MS due to its strong anti-inflammatory activity. It also improves MS-ge manifestations.

**Acknowledgements**

The author expresses her sincere gratitude to Prof. Luigi Aloe and Dr. Marco Fiore from the Institute of Neurobiology and Molecular Medicine, Rome, Italy, for their valuable help in the performance of the laboratory examinations, to Prof. Donald E. Greydanus from the Michigan State University, East Lansing & Kalamazoo, MI, USA, for his critical remarks and suggestions, and to Assoc. Prof. Dimitar Tomov from the Medical University of Varna, Varna, Bulgaria, for his help in the preparation of the manuscript.

**Conflict of interest statement:** The authors declare that they have no conflict of interest to the publication of this article.

**References**


**Table 3. Metabolism-related parameters in MS-ge before and after treatment with Mf alone and with Mf+NSAIDs**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MS-ge (n=20)</th>
<th>MS-ge (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before Mf</td>
<td>after Mf</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>42.1±0.3</td>
<td>40±0.3†</td>
</tr>
<tr>
<td>waist circumference (cm)</td>
<td>121.9±3.8</td>
<td>118.5±3.8</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>10.6±1.4</td>
<td>9.2±1.2</td>
</tr>
<tr>
<td>NGF (pg/mL)</td>
<td>21.4±16.8</td>
<td>15.7±9.9*</td>
</tr>
<tr>
<td>BDNF (pg/mL)</td>
<td>2721.1±878.1</td>
<td>2850.2±124.2</td>
</tr>
</tbody>
</table>

*p<0.05, †p<0.01, ‡p<0.001