Is Mycophenolate Mofetil an Alternative Agent to Corticosteroids in Traumatic Nerve Paralysis?

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Objective: The effects of an immunosuppressive agent, mycophenolate mofetil (MM), were investigated and compared with those of methylprednisolone (MP) and dexamethasone (DXM) on the traumatic nerve function.

Study Design: This is a randomized controlled animal study.

Materials and Methods: This experimental study was performed on 84 male Wistar albino rats. The rats were assigned to 12 groups each consisting of 7 animals. The groups were formed according to application of normal-dose DXM (group 1A-B), high-dose MP (group 2A-B), normal-dose MP (group 3A-B), MM (group 4A-B), and MM with high-dose MP combination therapies (group VA-B). Right sciatic nerve dissection was performed, and compound muscle action potential thresholds were recorded. The nerve was traumatized with the compression of a Jeweller forceps for 20 seconds. Posttraumatic thresholds were also recorded. The compound muscle action potential thresholds were recorded in the first and fourth weeks for the assigned groups. Then, the nerve was transected and prepared for electron microscopic and histopathologic examinations. Nitric oxide and malondialdehyde assessments were performed on both tissue and blood samples.

Results: Only the MM and MP+MM groups had satisfactory electron microscopic findings and were about to reach the tissue characteristics of the control animals. Despite the electrophysiologic recovery, the DXM group was found to have poor electron microscopic scoring.

Conclusions: Mycophenolate mofetil has been found to be beneficial in the treatment of traumatic nerve paralysis. Although a complementary investigation is needed, this immunosuppressive agent may be an alternative to corticosteroids for the selected cases where steroid therapy is contraindicated.

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Facial nerve paralysis, resulting with cosmetic and functional disorders, also leads to psychologic problems for the affected patients. These factors adversely affect their social lives and the quality of life.[1] Etiology, pathology, and disease management modalities of acute facial paralysis are still controversial. There is a wide variety of etiologic causes of this disease entity. The most common cause is the Bell palsy or idiopathic facial paralysis.[2,3] The second most common cause is trauma. There is still controversy on both the traumatic and non–traumatic facial paralysis regarding medical and surgical aspects.[4,5] Corticosteroids are widely used in Bell paralysis. Although the corticosteroids are shown to be the most effective drug group for medical management through meta-analysis,[6] they may cause many adverse effects including gastric ulcer, hypertension, osteoporosis, thrombophlebitis, and psychosis.[7] The current study investigates the effectiveness of mycophenolate mofetil (MM) as an alternative treatment to corticosteroids. Besides being used as an immunosuppressive agent, it is also used in myasthenia gravis, organ transplants, pemphigus vulgaris, multiple sclerosis, and polyarteritis nodosa. The comparison of the efficacy of MM to the corticosteroids is assessed through electrophysiologic, electron microscopic, histopathologic, and biochemical parameters. This study evaluates not only the effectiveness of MM but also the mechanism of action in light of the English literature.

MATERIALS AND METHODS

This experimental study has been performed in the biophysics laboratory in Mersin University medical faculty on 84 male Wistar albino rats each weighing 250 to 300 g. The experimental protocol was approved by the animal care and use committee of the University of Mersin (February 1, 2010). The groups were assigned as follows: group 1 (A, B), only dexamethasone (DXM) application (1 w and 1 mo, respectively); group 2 (A, B), only high-dose methylprednisolone (MP) application (1 w, 1 mo, respectively); group 3 (A, B), MP with dose reduction (1 w, 1 mo, respectively); group 4 (A, B), only MM application (1 w, 1 mo, respectively); group 5 (A, B), high-dose MP+MM application (1 w, 1 mo, respectively); group 6 (control); and group 7 (sham). The rats were anesthetized with 8 mg/100 g of ketamine and 1 mg/100 g of xylazine intraperitoneally. The right sciatic nerve was exposed at the right gluteal region in sterile conditions. After exposure of the sciatic nerve under an operating microscope, an initial electrophysiologic assessment was performed as described in the electrophysiologic assessment part of the Materials and Methods section. Then, the application of Jeweller forceps was performed 1 cm proximal to the trifurcation point of the sciatic nerve. The electrophysiologic assessment was performed after injury on the first and fourth weeks according to the groups.

Electrophysiologic Assessment

Data were collected by means of a BIOPAC MP100 acquisition system (Santa Barbara, CA). Bipolar surface electrodes (Medelec small bipolar nerve electrodes, 6894 T, Oxford, United Kingdom) were used for recordings from the gastrocnemius muscle. The ground electrode was placed on the thigh on the side of stimulation. The sciatic nerve was stimulated proximal to the lesion site by bipolar electrode. The supramaximal stimulus consisted of single square pulse (intensity, 0.5 V; duration, 0.2 ms). Compound muscle action potentials records were raised on the amplifiers and then transferred to the computer translating to the numerical signals by 16-bit analog-to-digital converter for an offline analysis. The sampling rate

was chosen as 20,000 samples per second. BIOPAC Acknowledge Analysis software (ACK 100 W) was used to measure amplitude of compound muscle action potential.

Biochemical Assessment

Nitrite-Nitrate Assay

The final products of nitric oxide (NO) in vivo are nitrite (NO_2^-) and nitrate (NO_3^-). The relative proportion of NO_2^- and NO_3^- is variable and cannot be predicted with certainty. Thus, the best index of total NO production is the sum of both NO_2^- and NO_3^-. The oxidized end products of NO (NO_2^- and NO_3^-) were measured in serum samples by the following assay. Measurements of NO_2^- and NO_3^- were made using a procedure based on the Griess reaction (9). The samples were obtained via indwelling catheters and immediately centrifuged at 4000 rpm for 10 minutes. The serum samples were ultrafiltrated and used in the test. Nitrates were quantitatively converted to NO_2^- for the analysis. Enzymatic reduction of NO_3^- to NO_2^- was carried out using enzyme cofactors in the presence of NO_3^- reductase. Enzyme and cofactors were added to each serum samples and standards. After a 1-hour incubation period at room temperature, Griess reagent was added, and all these mixtures were incubated for 10 minutes at room temperature in dimmed light. Then, the absorbance of the samples and standards were measured at 540 nm using a plate reader. In the measurement of total NO products, only the NO_3^- standard was required for standard curve preparation (Nitrate/Nitrite Colorimetric Assay Kit, 780001; Cayman Chemical Company, Ann Arbor, MI). Total levels of NO_2^- and NO_3^- were expressed as micromolar of serum.

Lipid Peroxide Assay

Levels of malondialdehyde (MDA), an index of lipid peroxidation, were determined by thiobarbituric acid reaction. The MDA levels in serum were determined by thiobarbituric acid reaction according to Yagi et al.[10] The principle of the method depends on the colorimetric measurement of the intensity of the pink color produced by the interaction of the barbituric acid with MDA. The colored reaction 1,1,3,3 tetraetoxypropane was used as the primary standard. The MDA levels were expressed as nanomolar per milliliter of serum.

Tissue Assessment

Nitrite-Nitrate Assay

The levels of NO were studied according to "vanadium-3-chloride-Gries reaction."

Lipid Peroxide Assay

The concentration of MDA was studied as described by Yagi et al.[8]

The Electron Microscopic Assessment

For transmission electron microscopic evaluation, the samples were fixed with 2.5% glutaraldehyde, postfixed with 1% osmium tetroxide, dehydrated in graded alcohol series, cleared with propylene oxide, and embedded in epon. Thin sections (50–70 nm) were cut using a microtome (Leica UCT-125) and contrasted with uranyl acetate and lead citrate. The sections were examined and photographed using an electron microscope (JEOL JEM-1011).

The Pathologic Assessment

In the laboratory of the Department of Pathology of Mersin University Medical School, the sciatic nerve samples were fixed in 10% buffered formaldehyde, embedded in paraffin, cut into 5-μm–thick sections, and mounted on slides. The deparaffinized sections were pretreated for citrate buffer, pH 6, and incubated with hydrogen peroxidase for 10 minutes. Hematoxylin-eosin staining was performed to all cases. Immunohistochemical staining was performed similarly for nerve growth factor receptor (NGFR) (ABBIOTEC, P75NTR) and vascular endothelial growth factor (VEGF) (DAKO, clone VG1, CODE NO. M7273) using the standard streptavidin biotin-immunoperoxidase method. Positive and negative controls were used for immunohistochemistry. The staining pattern was evaluated under the light microscope using Olympus BX50.

In the hematoxylin-eosin–stained sections, edema, fibrosis, as well as the number of neutrophils and lymphocytes were evaluated at the epineurium and were scored as follows: score 1, mild edema; score 2, moderate edema; and score 3, severe edema (for edema) as well as score 1, mild fibrosis; score 2, moderate fibrosis; and score 3, intense fibrosis (for fibrosis). The number of lymphocytes (counted 3 high power fields) scored between 1 and 3 (score 1, 0–2; score 2, 2–4; score 3, more than 4) and the number of neutrophils (counted 3 high power fields) scored between 1 and 3 (score 1, 0–2; score 2, 2–4; score 3, more than 4). For the immunohistochemical evaluation of NGFR and VEGF, the following parameters were used: the intensity of staining of Schwann cells in the endoneurium, the intensity of staining in vascular structures in the epineurium and endoneurium, as well as the intensity of staining of fibroblasts in the endoneurium, epineurium, and perineurium. The density of staining was scored between 0 and 3 for both NGFR and VEGF: score 0, no staining; score 1, weak staining; score 2, moderate staining; and score 3, intense staining.

RESULTS

Groups 1, 4, and 5 showed statistically significant recovery in amplitudes in the first month values when compared with those of the first week (Fig. 1).

The mean amplitude values of all the groups except group 3B almost reached to the value of the control animals. In terms of electron microscopy, the greatest similarity to the controls was found in group 4B (Fig. 2).

Although the mean amplitude of group 1 was found to be in a very good level, the electron microscopic findings were found to be poor compared with those of the control group (Fig. 3). Interestingly, in both the serum and the tissue NO, MDA values were found to be strongly correlated (Fig. 4).

The animals in the MM group showed intense VEGF and nerve growth factor (NGF) staining compared with those of the controls (Fig. 5). The correlation of the edema parameter of the nerve to both

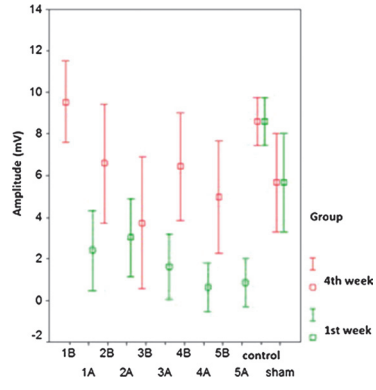

FIGURE 1. Amplitudes of all groups in the first month values when compared with the first week levels.

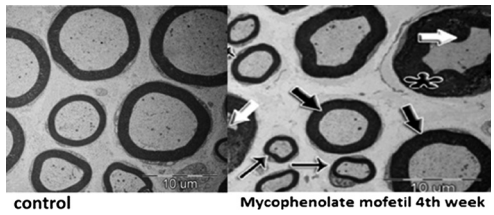

FIGURE 2. In terms of electron microscopy, the greatest similarity to the controls was found in group 4B. Thick black arrow shows normal myelinated axons; thin black arrow, newly formed myelinated axons; white arrow, ovoid formation of myelin; asterisk, heavy delamination and disintegration in myelin sheath.

the amplitude and the latency was found to be statistically significant ($P < 0.05$).

DISCUSSION

The current study evaluated the efficacy of MM as a protectant agent against traumatic sciatic nerve injury in the rats. The neurophysiologic, electron microscopic, or biochemical assessments are widely taken into consideration in various studies investigating the neural tissues after various experimental insults. There is still a controversy of investigations aiming to study the previously mentioned factors altogether. The current study aimed to investigate all the 3 mentioned parameters and the relationship among them.

Our study demonstrated that the best electrophysiologic results, in terms of amplitudes, have been seen in the DXM group. The high-dose MP group and the MM group also showed good mean amplitude levels. We could not find a very high correlation between the electrophysiologic status and the electron microscopic findings in the DXM group.

After the injury, the inflammatory process is to be established in a balanced manner for the healing of the nerve tissue. The formation of excessive inflammatory reactions causes the occurrence of dense fibrous connective tissue leading to scar formation.[9] The previously mentioned ongoing process disrupts the regeneration of the normal spatial organization of the nerve. Corticosteroids and the nonsteroid anti-inflammatory drugs are reported to be useful in the prevention of excessive fibrosis formation.[10]

One of the most commonly used and accepted drugs in the treatment of traumatic nerve paralysis is the corticosteroid group. Corticosteroids reduce the posttraumatic capillary permeability with the reduction of the interfascicular edema. A lower degree of nerve compression contributes to milder nerve degeneration, and higher regeneration results in reduced lipid peroxidation.[11]

The current study revealed that, although DXM provided a good electrophysiologic condition, histopathologic findings were not as good. Discordance is reported between electrophysiologic and electron microscopic findings in various studies performed on this topic.[12,13]

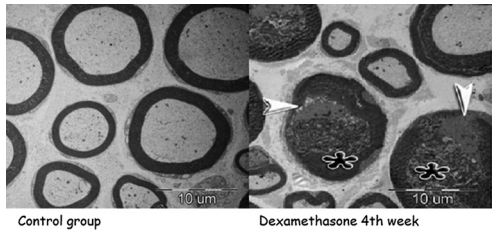

FIGURE 3. The worst electron microscopic finding is seen in the DXM group. White arrowhead shows darkening and loss of axoplasm; asterisk, heavy delamination and disintegration in myelin sheath.

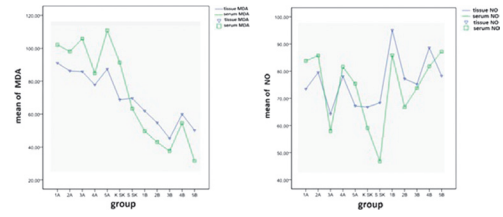

FIGURE 4. In both the serum and the tissue nitric oxide, malondialdehyde values were found to be strongly correlated.

This discrepancy was attributed to the effect of this drug, which decreases the interfascicular edema, but far from establishing an adequate degree of nerve regeneration. This finding is reported in other electrophysiologic studies.[12]

We have achieved sufficient improvement electrophysiologically in terms of amplitude but have not obtained such adequate success electron microscopically. Therefore, a longer duration of investigation for approximately 8 to 12 weeks is to be planned on this topic to achieve a more detailed and comprehensive ultrastructural healing outcome.

The *oxidative stress* is defined as the increased formation of reactive oxygen species and decreased antioxidant defense mechanism. It is recognized as an important feature of various diseases such as nerve injury. Lipid peroxidation is one of the most important expressions of oxidative stress induced by reactive oxygen species, and MDA is an indicator of lipid peroxidation.[14,15] The significant decline of mean MDA levels toward the end of the first month can be interpreted as the recovery of the degenerative process.[16] Nitric oxide is an important signaling molecule that acts in many tissues to regulate a diverse range of physiologic processes. The production of NO from L-arginine has been reported to play a significant role on wound healing processes. NO is mediating synaptic plasticity as well as neural signaling and may play an important in neural injury. Peak levels of NO were seen during the first day; their decline, during the third and fifth days.[14,15]

We have noticed significant correlation between serum and tissue MDA levels. There is a fact that performing a biopsy from a nerve is not logical in clinics. Therefore, although tissue examination seems more appropriate, serum studies of NO and MDA would be helpful to interpret the recovery phase of the nerve. This correlation deserves attention for serum values for follow-up of patients.

NGF supports the life of nerve cells and the growth of the nerve fibers. It is known that Schwann cells secrete NGF after nerve

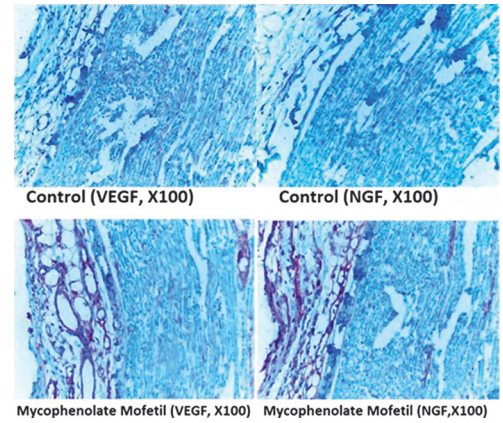

FIGURE 5. The animals in the MM group showed intense VEGF and nerve growth factor staining compared with those of the controls.

injury.[17,18] Vascular endothelial growth factor mainly increases permeability, growth, as well as migration of endothelial cells and stimulates angiogenesis.[19] Histopathologic examination results revealed that VEGF and NGF staining was found to be much more pronounced and intense in the 2B and 4B groups compared with those of the controls. These findings seem to support regeneration in the high-dose MP and MM groups.

CONCLUSIONS

Mycophenolate mofetil is an immunosuppressive agent that has tolerable gastrointestinal adverse effects. The current study revealed that this drug provided a satisfactory electrophysiologic improvement after nerve injury. The administration of MM alone or together with MP may be an appropriate option in the treatment of peripheral nerve paralysis. It might be an alternative where corticosteroid therapy is contraindicated or avoided for their serious adverse effects.

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