Ocular Neostigmine Drops for Diagnosing Myasthenia Gravis

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Abstract

Introduction: A variety of tests have been devised for the diagnosis of myasthenia gravis (MG). The best known of these (Tensilon test, using intravenous edrophonium chloride) may cause serious complications (bradycardia and syncope) dictating cardiac monitoring during the procedure. Ocular neostigmine drops, a simple procedure, may significantly reduce the risk of diagnostic testing for possible MG.

Method: To investigate its efficacy, the miotic effect of neostigmine was explored using 30 rabbits. One drop of sterile neostigmine solution (2.5 mg/ml) was instilled into the right eye (RE) of each rabbit using the left eye (LE), which received sterile normal saline, as control. Serial assessments of pupillary size were done. One drop of neostigmine was instilled daily for 7 days to investigate its safety. Six patients (aged 4.5–55y, median=42y, mean=36.4y) with MG had the same test and were observed for increase of the palpebral fissure height (documented by photography).

Results: At baseline there was no significant difference in the mean [SD] pupillary size of the rabbits between the RE (7 mm [1.07]) and LE (7 mm [1.07]), p=0.63. Significant miotic effect was observed in the RE compared to the LE at 30, 60 and 90 minutes (respectively, 4.8 mm [1.86] vs 7.0 mm [1.09], p=0.0001; 4.8 mm [1.86] vs 7.0 mm [1.09], p=0.0001; and 3.2 [0.76] vs 7.0 [0.10], p=0.013). Administration of one drop of neostigmine daily for 7 days caused no ocular inflammation. All six patients with MG had an observable increase of the palpebral fissure height (documented by photography) of at least 2 mm, 30 minutes after neostigmine instillation. The response was dramatic in the three patients with no prior treatment for MG.

Conclusion: Ocular neostigmine drops is a safe, simple and efficient diagnostic test for MG.

Keywords: Myasthenia gravis; Diagnosis; Ocular neostigmine drops; Tensilon test; Edrophonium chloride

Introduction

Myasthenia gravis (MG) is an acquired autoimmune disorder caused by autoantibodies directed against epitopes on or around the acetylcholine receptor (AChR) in the muscle membrane [1]. The condition is characterized clinically by variable ptosis, ocular motility abnormalities, and weakness of voluntary muscles in other parts of the body [1]. Nearly all patients present with extraocular muscles involvement, causing ptosis and diplopia, and in 20–40% of patients, weakness remains limited to these muscles (ocular MG) [1-3]. The diagnosis may be difficult to make on clinical grounds especially in juvenile cases when weakness is restricted to few muscles such as in ocular MG [4], and a variety of supportive diagnostic tests have been devised [5].

Perhaps the best known of these is the Tensilon test [5-8], using intravenous edrophonium chloride, which is an acetylcholinesterase (AChE) inhibitor with very short action. Unfortunately, edrophonium causes cholinergic side effects that may include increased oral and bronchial secretions, Bradycardia, abdominal cramps, sweating, heart block, hypotension, and red, watery, painful eyes. These are often uncomfortable for the patient, and although serious complications of Bradycardia and syncope are rare [9] the test is now commonly performed with cardiac monitoring and atropine available as an anti-cholinergic agent. Other relative contraindications for performance of edrophonium are cardiac dysrhythmias and bronchial asthma. A second quick bedside test is by applying an ice pack locally to a ptotic eyelid for 2-5 minutes and observing for any improvement of ptosis or eye movement deficit [10-12]. The ice pack test is simple, inexpensive, and has a reported sensitivity of 93–97% and specificity of 97–98% [13]. This adjunctive diagnostic test is particularly helpful if the edrophonium test is contraindicated or not available. However, the procedure is unlikely to be tolerated by young children [14].

Other supportive tests require laboratory and electrophysiologic facilities. Serologic tests include assessing AChR antibodies which are found in 80% of adults with MG [15], 70% of peripubertal juvenile MG but only 50% of prepubertal MG [16,17]. Antibodies to muscle-specific kinase (MuSK) are rare in juvenile MG [18].

Electrodiagnosis of MG includes repetitive nerve stimulation [RNS] (looking for a decrement of greater than 10% of the fifth compared to the first-evoked compound muscle action potential [CMAP] in...
The left eye of each rabbit acted as control for the right one. A solution of sterile saline (0.9% sodium chloride) was used in an eye, limiting substantially the amount of drug that might be absorbed systemically. The sterile neostigmine solution (2.5 mg/ml) was instilled into the right eye (one drop), while the control eye (left eye) received one drop of saline. Single-fiber electromyography (SFEMG) requires a significant degree of cooperation and expertise [18] and is not available in many hospitals. In young children, sedation may be required to facilitate both RNS and stimulated SFEMG [19]. An additional simple, safe, sensitive, and specific diagnostic test for MG is, therefore, needed.

Methods

This research project investigated the safety and efficacy of using intravenous (IV) neostigmine solution, available in all hospitals where anesthesia is performed, as an ocular drop for diagnostic purposes in the setting of patients with probable or definite autoimmune MG.

The study had two components: 1) experiments using 30 rabbits in which the standard IV neostigmine solution was instilled into one eye (using the other as control) to evaluate effectiveness (i.e., changes in the pupillary size of the experimental eye) and safety (i.e., does not cause significant irritation of the experimental eye or obvious systemic effects). 2) experiments in which the standard IV neostigmine solution was instilled into both eyes of patients with probable or definite symptomatic MG.

Both sets of experiments used the standard neostigmine (2.5 mg/ml) solution available in hospitals for anesthetic/surgical use for reversal of neuromuscular block. This solution is buffered and FDA-approved for use in humans. In both sets of experiments, one drop of this solution was used in an eye, limiting substantially the amount of drug that might be absorbed systemically.

Rabbit studies

Male New Zealand White rabbits (2.5–3.0 kg body weight) were used in all experiments. The animals were kept in a room of controlled room temperature (24+1.0°C) and humidity and a fixed dark light cycle (light on at 7:00 am and off at 19:00 pm).

The sterile neostigmine solution (2.5 mg/ml) was instilled into the right eye (one drop), while the control eye (left eye) received one drop of sterile saline (0.9% sodium chloride). Assessment of pupillary size and LE (7 mm [1.07], p=0.63). Significant miotic effect was observed in the RE compared to the LE at 30 and 60 minutes (respectively, 4.8 mm [1.86] vs 7.0 mm [1.09], p=0.0001; and 4.8 mm [1.86] vs 7.0 mm [1.09], p=0.0001).

Results

Human studies

Patients with probable or definite MG were asked to sign informed consent approved by the appropriate Institutional Ethics Committees. Ptosis was recorded photographically, and pulse and blood pressure were also recorded prior to beginning the test. One drop of standard IV neostigmine solution (2.5 mg/ml) was instilled into each symptomatic eye of the patient. Fifteen minutes later, possible changes in ptosis were assessed. Pulse and blood pressure was recorded again and after another 15 minutes. At 30 minutes, the patient was evaluated also for possible changes in ptosis (documented by photography), obvious irritation of either eye and for report of discomfort.

Rabbit studies

At baseline (Figure 1) there was no significant difference in the mean [SD] pupillary size of the rabbits between the RE (7 mm [1.07]) and LE (7 mm [1.07], p=0.63). Significant miotic effect was observed in the RE compared to the LE at 30 and 60 minutes (respectively, 4.8 mm [1.86] vs 7.0 mm [1.09], p=0.0001; and 4.8 mm [1.86] vs 7.0 mm [1.09], p=0.0001).

The amount of neostigmine methylsulphate was calculated to be 0.1 mg/drop. Therefore, each rabbit’s eye was instilled by 0.1 mg of sterile neostigmine methylsulphate. The sterile dropper for instilling neostigmine was used only once and then discarded. To prevent the drop of neostigmine from leaking out of the rabbit’s eye, the lower lid of the rabbits was held out for at least 2–3 minutes in order to keep the drop inside the eye and allow for better absorption of the drug through the cornea.

The pupil size of both the right and left eyes of each rabbit was measured at baseline in mms by using a plastic ruler. After instilling the neostigmine solution (right eye) or saline (left eye), the pupillary size was measured again at 30 minute intervals following drug or saline instillation. One drop of neostigmine was instilled daily for 7 days and signs of ocular inflammation were observed.

Statistical analysis: The mean values for the pupil sizes of the right eyes were recorded as well as those of the left eyes, separately. Paired t-test was used for comparison of data among the different groups on SPSS software. A probability value of <0.05 was considered to be significant.

Figure 1: Mean pupil diameter measurements of the right and left eyes of 30 rabbits over time. One drop of neostigmine solution (2.5 mg/ml) was instilled in the right eye, whereas one drop of saline was instilled in the left (control) eye.
MG appears to be relatively high in Asians [21]. Also in a series from older age group [20]. The proportion of childhood-onset (<15 years) peaking between 60 and 80 years, being more predominant in the both sexes, the incidence rate was reported to increase with age, year and prevalence rate ranging from 15 to 179 per million [20]. Incidence rate ranging from 1.7 to 21.3 cases per million person per 1, 2 and 5).

Patients

Six patients (3 males and 3 females) who presented with ptosis and/or ocular motility disturbance were enrolled in the study (Table 1). Their ages ranged between 4.5 and 55 years (median=42 years, mean=36.4 years). The duration of their symptoms ranged between 1 and 18 months (median=4 months, mean=5.8 months). Three of them were not suspected before or investigated for MG, and hence received no treatment (Patients 1, 2 and 5). The other 3 (Patients 3, 4 and 6) were seropositive for AChR-antibodies, and one (Patient 6) had, as well, significant decrement (>10%) on repetitive nerve stimulation (RNS). Patient 3 (Table 1) had prednisone and pyridostigmine for 3 months, another (Patients 4) had prednisone for 2 months, and a third (Patient 6) had pyridostigmine for one year. Patient 3 received 30 mg of pyridostigmine 2 hours prior to the test whereas Patient 6 had 60 mg of pyridostigmine 8 hours earlier. The 6 patients manifested clinically with ocular MG, with no limb, axial muscles, oropharyngeal or respiratory muscle involvement [5].

All 6 patients with MG had an observable increase of the palpebral fissure height (documented by photography) of at least 2 mm, 30 minutes after neostigmine instillation (Figure 3). The response was dramatic in the 3 patients with no prior treatment for MG (Patients 1, 2 and 5).

Discussion

Population based epidemiological studies in MG reported an incidence rate ranging from 1.7 to 21.3 cases per million person per year and prevalence rate ranging from 15 to 179 per million [20]. In both sexes, the incidence rate was reported to increase with age, peaking between 60 and 80 years, being more predominant in the older age group [20]. The proportion of childhood-onset (<15 years) MG appears to be relatively high in Asians [21]. Also in a series from Saudi Arabia [22], children (≤ 16 years) accounted for 20% of 150 patients who had thymectomy for the treatment of MG. The incidence of juvenile patients who present with ocular MG has been reported to range from 27% to 93%, with substantially high incidence of ocular presentation in Asian children [18]. A trend towards developing ocular MG rather than generalized MG was reported from South Africa in children of African ancestry [23], with an age peak between 2 and 4 years [24]. It's noteworthy that under-recognition of MG is likely in Developing Countries where access to specialist services is poor [25].

Although the clinical course of MG is variable, spontaneous remission is still uncommon and the time of diagnosis from onset (<1 year) and intervention was reported to be the most robust prognostic factor for remission [26]. Nevertheless, clinical diagnosis may be difficult especially when weakness is restricted to few muscles such as ocular MG.

The two bedside tests with high sensitivity and specificity include the ice pack and edrophonium tests. Nevertheless, application of ice over the eyelid for 2-5 minutes is unlikely to be tolerated by children (personal observation). Edrophonium test, which requires IV infusion of the AChE inhibitor edrophonium chloride, has a reported sensitivity of 71.5-95% and specificity of 97% [2,27]. The sensitivity of the test is likely to be lower in the busy clinical practice [2]. Moreover, false-positive tests (which have been reported with Guillain-Barre syndrome, brain stem lesions and compressive cranial neuropathies) might be higher in these settings [2,27]. Due to the potential serious cholinergic effects, edrophonium test in children should be conducted in a monitored setting with availability of atropine and staff trained in cardiac resuscitation [1,18,19]. Some patients with congenital myasthenic syndromes may deteriorate when exposed to an AChE inhibitor such as COLQ and MuSK mutations [28,29]. Also edrophonium is not

<table>
<thead>
<tr>
<th>Patient (P) No.</th>
<th>Gender</th>
<th>Age</th>
<th>Duration of symptoms</th>
<th>Diagnostic test (s)</th>
<th>Treatment prior to ocular neostigmine (duration)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 P2 P3 P4 P5 P6</td>
<td>M M F F M F</td>
<td>56y 37y 4½ y 48y 27y 47y</td>
<td>3 mo 1 mo 5 mo 5 mo 3 y 1½ y</td>
<td>ND Positive AChR antibody Positive AChR antibody ND Positive AChR antibody</td>
<td>None None Prednisolone and pyridostigmine (3 mo) Prednisone (2 mo) None</td>
</tr>
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Abbreviations: AChR: Acetylcholine Receptor; F: Female; M: Male; mo: month; ND: Not Done; RNS: Repetitive Nerve Stimulation; y: years.

Figure 2: Pupillary response to instillation of neostigmine (right eye) and saline (left eye). Images A-D=right eye; Images E-H=left eye. A=Pre neostigmine (6.0mm); B=30 mins post neostigmine (2.0mm); C=60 mins post neostigmine (1.5mm); D=1 week after daily instillation of 1 drop of neostigmine, last drop 24 hours previously (6.0mm); E=Pre saline (7.0mm); F=30 mins post saline drop (7.5mm); G=60 mins post saline drop (7.0mm); H=1 week after daily instillation of 1 drop of saline, last drop 24 hours previously (7.0mm).

Figure 3: Ocular neostigmine test in 6 patients with myasthenia gravis (MG). (A, C, E, G, I, K) baseline ptosis in Patients 1-6, respectively. (B, D, F, H, J, L) Observable increase of the palpebral fissure 30 minutes after instillation of neostigmine drops. The response was striking in Patients 1, 2 and 5 (respectively, B, D and J) who had not received treatment for MG.

Figure 4: Representative photograph of these pupillary changes is shown in Figure 2.
readily available in many hospitals of the Developing Countries. This is what prompted one of the of Authors (Mo A S) during his work in a district hospital in Sudan, to pioneer the idea of ocular neostigmine as a diagnostic test for MG. The neostigmine preparation (2.5 mg/ml) which has been used as ocular drops in the present study is available as IV solution (2.5 mg/ml) in all hospitals where anesthesia is performed.

The rabbits were chosen to test for the efficacy and safety of neostigmine eye drops since they are established experimental model for research on MG [30,31]. The rabbit experiment was included to show that the concentration of the drug is in the correct range, and that the drug does not cause irritation. Significant mictic effect of ocular neostigmine could be demonstrated at 30 and 60 minutes following its instillation (Figures 1 and 2). This significant mictic effect was also evident at 90 minutes. No ocular inflammation was observed even after administration of neostigmine daily for 7 days.

The application of neostigmine drops for 3 patients, who received no prior treatment for MG because the disease has not been suspected before, showed dramatic response (Figure 3). It has been reported that MG is likely to remain restricted to the ocular muscles if the patient had restricted ocular manifestation of the disease for more than 2 years, similar to what has been observed in Patient 5 (Table 1) who had ocular MG for duration of 3 years [2]. The other patients (Table 1), who were all seropositive for AChR antibody, had prior treatment for MG (prednisone and/or pyridostigmine) and the disease has also been confirmed by significant decrement on RNS in a 47-year-old female (Patient 6, Table 1) who had the longest duration of therapy (pyridostigmine for one year). Although the response to neostigmine drops was less dramatic than in the first 3 patients who received no treatment for MG, an observable increase of the palpebral fissure height of at least 2 mm was observed 30 minutes after neostigmine instillation (Figure 3). This sub-group included a 4½-year-old girl in whom edrophonium test is expected to be met with technical difficulties, as well as safety reservations. No ocular or systemic side effects were observed in any of the 6 patients.

**Conclusions**

Based on the present experimental and clinical research, ocular neostigmine drops proved to be an alternative safe, simple and efficient diagnostic test for MG. It may fulfill the need in many regions around the world where the other standard tests for MG are either unavailable or technically not feasible.

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**References**