THE DIAGNOSIS AND TREATMENT OF ACQUIRED MYASTHENIA GRAVIS IN TWO ADULT DOGS USING ORAL NEOSTIGMINE BROMIDE


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Summary: Two adult dogs aged 3.5 and 9 years were diagnosed with acquired Myasthenia Gravis (MG) based on clinical signs and positive response to oral neostigmine bromide (0.25mg/Kg BW) within 4 hours of oral administration. Both patients were successfully treated using neostigmine (1mg/Kg BW daily) alone or with neostigmine (1mg/Kg BW daily) and prednisolone (1mg/Kg BW, q12h) combination. Serum alkaline phosphatase and albumin/globulin ratio monitored in one of the above patients starting from one month prior to development of generalized clinical signs until complete recovery, showed significant alterations which can be used in diagnosing MG and in monitoring the response to treatment.

BACKGROUND

Myasthenia gravis (MG) is a disorder of neuromuscular transmission manifested by weakness of skeletal muscles (Drachman, 1978; Shelton, 1999). There are two forms of the disease namely congenital and acquired. Congenital MG (CMG) is due to deficiency or functional disorder of the nicotinic acetylcholine receptor (AChR) located at the neuromuscular junction of skeletal muscles whereas the acquired MG (AMG) is an immune mediated disorder in which auto antibodies are produced against AChRs (Dewey et al., 1997; Stenner et al., 2003; Webb et al., 1997). CMG is a rare disorder that has been reported only in few breeds including Russell Terriers, English Springer Spaniels, Smooth Fox Terriers and Smooth-haired miniature Dachshund. The autoimmune AMG appears to be relatively common in dogs and is reported in cats and is very similar to MG in humans.

The AMG affects adult dogs and appears to have peak incidences in two age groups; 2-4 years and 9-13 years (Tilley and Smith Jr, 2015). Generalised, focal and acute fulminating forms of MG have been described in dogs (Stenner et al., 2003). Focal form may appear in variable sites including pharyngeal, laryngeal, oesophageal or ocular muscles. Characteristic clinical features of generalized form include progressive appendicular weakness (that deteriorates with exercises and improves with rest), weakness of the facial and extraocular muscles, difficulty in swallowing, and aspiration pneumonia secondary to megaesophagus and regurgitation (Garlepp et al., 1984). The acute fulminating MG is characterized by sudden onset of megaesophagus and generalized muscle weakness leading to recumbency within days (King and Vite, 1998). Megaesophagus is common in dogs with MG since the oesophagus largely consisted of skeletal muscles (Wray and Sparkes, 2006). In addition, AMG has been reported to occur as a paraneoplastic syndrome in association with thymic neoplasia in some dogs (Rusbridge et al., 1996).

This communication describes acquired generalized MG in two adult dogs presented to the Veterinary Teaching Hospital (VTH) Peradeniya, diagnosed using oral neostigmine test and treated successfully with neostigmine.

CASE REPORTS

Case 1: A three and half year old neutered female Labrador retriever (patient “A”) was presented with knuckling right hind paw and inability to bear weight on both hind limbs and was reported to have acutely become deaf two months ago. She had been brought to the VTH a month ago due to reduced activity and appetite, and mild diarrhoea. Serum biochemistry profiles on the first visit showed highly elevated alkaline phosphatase (ALP: 1914 U/L, Normal range: 22 to 143 U/L) with normal alanine transaminase (ALT: 110, Normal range: 5-
107 U/L) and aspartate transaminase (AST: 34.9, normal range: 5-55) levels and was treated for suspected gall stones with oral ursodiol (a bile acid). She was brought to the VTH 28 days after the first visit due to non productive cough and inappetence. Pleural and pericardial effusions were detected with ultra sound scanning and was diagnosed as right sided heart failure and treated with furosemide (80mg, bid), spironolactone (75mg, bid) and enalapril (10mg, bid). Two days later, she had started limping on right hind limb, reluctant to stand up and move, therefore was brought back to the VTH. She was bright and alert with good appetite and normal body temperature (101.4 °F), normal heart rate (80bpm) and respiratory rate (36 /min) at the time of presentation. Her spinal reflexes were adequate, except for the absence of pedal reflex in right hind limb. She showed deafness both in the right and left sides. The differential diagnosis for generalized appendicular weakness included spinal injury, intervertebral disk disease, hypokalaemia, metabolic diseases such as hypothyroidism or hypoadrenocorticism and generalized myasthenia gravis. In order to identify the cause, the previously prescribed medications were discontinued and the patient was closely monitored in the continuous monitoring unit (CMU) while performing further investigations.

On the first day of admission to the CMU normal erythrogram, leukogram with lymphocytosis (6.1x10³/µl) and hypokalemic (3.6mEq/L, normal: 4.37-5.35mEq/L) were evident. Thoracic radiographs taken on admission ruled out megaesophagus while there were evidence of alveolar pattern (air in alveoli is replaced by fluid or cells) on several lung lobes. A moderate increase in soft tissue opacity in the caudo-dorsal lung field was suggestive of pulmonary oedema. Abdominal ultrasonography showed slight hepatomegaly and splenomegaly.

On second day of hospitalisation, her heart rate and respiratory rates increased (108 bpm and 48/min, respectively) and was unable to stand up. The second thoracic radiograph showed further progression of pulmonary pathology, and broad spectrum antimicrobial therapy (Amoxicillin-clavulanate; 20mg/kg BW, IV, q12h) and aminophylline (10mg/kg BW, IV, q12h) were initiated. Potassium chloride (3mmol/kg BW daily) was given as a slow infusion to correct the hypokalaemia.

On the following day (Day 3 CMU) she had a grade 3 non-ambulatory paraparesis (able to move legs and wag tail, but was not strong enough to support the weight and walk), severe dyspnoea, orthopnoea and inspiratory crackles on auscultation.

Serum biochemical tests performed on the day 4 of the admission (labelled Day 35 in the course of MG in Figures 1a and 1b) showed elevated ALP (329.5 U/L) with normal ALT levels and low albumin/globulin ratio (A/G: 0.31, normal 0.8-2.0). In the absence of significant clinical improvement with symptomatic treatment given, a high dose of methylprednisolone sodium succinate (30mg/Kg BW IV) was administered intravenously, as laboratory findings were suggestive of an immune mediated disease. However, her condition deteriorated further and she remained recumbent and showed hyperesthesia with vocalization even on a gentle touch.

In order to confirm whether the generalized muscle weakness was due to myasthenia gravis, oral neostigmine test was performed on the 6th day; neostigmine bromide tablet (15mg) at a dose rate of 0.25mg/ Kg, BW orally and observed the patient for four hours for any change in the clinical signs. Within 2 hours of oral neostigmine, her exaggerated pain reaction and vocalization diminished and she was able to lift the head up, therefore, oral neostigmine treatment was initiated (2mg/kg BW, daily in four divided doses). Within 48 hours of treatment she was able to move the pelvic limbs. On the 9th day of hospitalization she stood up but the forelimbs were slightly weak. Adverse cholinergic reaction, characterized by muscle twitching around head and neck and diarrhoea was observed on the 12th day of hospitalization; thus, the dose rate was reduced to 1mg/kg BW daily. However, the muscle twitching and diarrhoea did not resolve completely and the muscles of the forelimbs did not regain the expected tone. Therefore, on 15th day of hospitalization prednisolone (1mg/kg BW, q12h) was given orally with a reduced dose of neostigmine (0.5mg/kg BW daily in four divided doses). On the 22nd day of hospitalization she became completely ambulatory. She was prescribed neostigmine and prednisolone for 7 more days and was discharged from VTH.

The serum ALP, ALT and albumin globulin ratio (A/G) of above patient was analysed from one month prior to development of generalized clinical signs to the complete recovery. As shown in the Figure 1a, her serum ALP level was elevated at the time of developing non specific signs while ALT and AST (measured only on the first day and not included in the graph) were within normal ranges.
Figure 1a: Variations in serum Alkaline Phosphatase (ALP) and Alanine Transferase (ALT) in patient “A” during the course of myasthenia gravis

Figure 1b: Variations in Total Protein, Albumin, Globulin and albumin/Globulin ratio of the patient ‘A” during the course of myasthenia gravis

Day 1: Onset of none specific clinical signs (reduced activity, appetite and mild diarrhoea)
Day 35: Knuckling on right hind limb, hind limb weakness (4th day of hospitalization)
Day 42: After high dose of IV methyl prednisolone and oral neostigmine
Day 64: On completion of three week course of oral neostigmine

Case 2: An eight year old cross bred intact male dog (patient “B”) was presented recumbent, coughing, severely dyspnoeic and cyanosed, and hence admitted directly to the intensive care unit (ICU) of VTH for oxygen and intravenous fluid therapy.

The temperature of the patient was normal (101.7 °F) and he showed generalized weakness with the pelvic limbs being more severely affected. The panniculur, pedal, anal and palpebral reflexes were intact. Thoracic auscultation revealed increased bronchovesicular sounds, laboured breathing and cardiac arrhythmia. The thoracic radiographs showed evidence of pneumonia and mild megaesophagus. Therefore, intravenous clavulanated amoxicillin (22mg/kg BW q 12h), aminophylline (200mg, q12h) and chlorpheniramine maleate (4mg twice daily) were administered. Since appendicular weakness and megaesophagus are commonly seen in MG patients, oral neostigmine test was performed. The patient responded well to neostigmine and was able to stand and walk a few feet within four hours. Following two doses of oral neostigmine, his condition improved remarkably. Once he started to show adverse cholinergic reaction of diarrhoea and hyper salivation, the neostigmine dose was reduced to 1mg/kg BW in four divided doses. On the 5th day
of hospitalization, he recovered from clinical MG and was discharged from the hospital with neostigmine (1mg/kg BW daily) prescribed for two weeks. After two weeks of neostigmine therapy, signs of muscle weakness and knuckling have been observed by the owner in the right forelimb. Therefore, neostigmine therapy was continued for two more weeks and no relapse of signs was reported thereafter.

**DISCUSSION**

Acquired myasthenia gravis occurs due to immune-mediated destruction of postsynaptic nicotinic acetylcholine receptors in skeletal muscles impairing neuromuscular transmission, which is clinically manifested as weakness in skeletal muscles. This communication discusses the disease progression of acquired MG in two canine patients starting from non-specific clinical findings that had not initially been attributed to myasthenia gravis. The significance of serum ALP and A/G ratio in diagnosing MG and monitoring the response to treatment have been emphasized. The use of oral neostigmine bromide for diagnosis and successful treatment of acquired MG in canine patients is also highlighted.

During the assessment of the patient “A”, the loss of hearing was initially thought to be a result of ototoxicity secondary to previous medications given. However, sudden sensorineural hearing loss has been observed in patients with autoimmune diseases such as MG, systemic lupus erythematosus (SLE) and Guillain-Barre like syndromes. The affected patients show hearing loss particularly to high frequency waves (above 30 decibels). A number of human studies have shown progression of MG associated with irreversible cochlear damage causing sudden loss in hearing (Arnold, 1997; Hamed et al., 2006). MG could cause non suppurrative otitis media, leading to hearing loss (Brookler et al., 1972). Many patients have progressed to generalize MG after focal MG in ocular, facial, tongue, oropharyngeal or oesophageal muscles. Even though sudden loss of hearing is not normally considered as an early sign of MG, it is important to note that acute deafness in patient “A” was observed before signs of generalized MG developed.

Both patients showed severe respiratory distress with the progression of the disease. Difficulty in breathing is a common clinical sign of MG, mainly due to the accumulation of secretions in the air ways. These secretions are usually removed from airways by coughing but MG patients are unable to cough up effectively due to weaknesses in expiratory muscles. In addition, intercostal and diaphragmatic muscles needed for inspiration, may not be strong enough to create an adequate negative inspiratory force or vital capacity (Keesey, 2004). MG patients with megaesophagus often develop pneumonia secondary to aspiration. Therefore, the severe respiratory distress seen in patient “B” by the time of admission might be partly due to megaesophagus. It is clear that the MG patients are more prone to develop pneumonia due to one or more of the above reasons. Therefore, broad spectrum antimicrobial(s) and bronchodilators should also be included in the treatment plan.

The diagnostic significance of elevated serum ALP levels detected in patient “A” is a noteworthy observation. The ALP comprises a heterogeneous group of enzymes from the kidney, liver, bone, placenta, and intestine of dogs. Isoforms from the intestine, kidney, and placenta are not detected in the serum due to their short half-life. Hepatic ALP is mostly represented near the canalicular membrane of the hepatocyte. Accordingly, bile-duct obstruction, primary sclerosing cholangitis, and primary biliary cirrhosis (PBC) are some examples of diseases in which ALP elevates. Interestingly, elevated ALP may also reflect the abnormal activation of T lymphocytes in autoimmune diseases (Hanna et al., 1997). Furthermore, this patient showed a very low A/G ratio indicating overproduction of globulins which is often noticed in autoimmune diseases.

While laboratory and bed side tests can be used to diagnose acquired MG, the demonstration of serum autoantibodies against AChR is considered the gold standard (King and Vite, 1998). Circulating antibodies that bind to the receptors are quantified using an immunoprecipitation radioimmunoassay using 125I-bungarotoxin labeled canine AChR and an antibody concentration ≥0.6 nmol/L is considered positive (Otte et al., 2003). Since serological testing to detect autoantibody level was not available in the patients described here, the actual pathogenesis of MG remains unknown. A positive response to ultra-short acting anticholinesterase agent; edrophonium chloride (0.1-0.2mg/Kg BW IV) is also being used as a presumptive diagnosis of MG, where a positive response is indicated by dramatic improvement in muscle strength within few minutes of administration (Otte et al., 2003).

Intravenous or intramuscular neostigmine bromide, a longer acting AChE, combined with atropine followed by 1 hour observation for improvement have also been used to diagnose MG (Namba et al., 1970). Oral neostigmine test is an alternative to IV and IM neostigmine tests (Tether, 1948). Human patients are given 60 mg of neostigmine orally and monitored for 90 minutes. As the dose rate for this therapeutic test for dog was not established, we used neostigmine in 0.25mg/ Kg BW. Diagnosis was established by improvements in muscle strength or alleviation of
hyperesthesia within four hours of oral neostigmine therapy.

Glucocorticoids are normally used to treat human MG patients. High dose of intravenous methylprednisolone have been reported to produce rapid improvement in acquired MG patients. However, an initial worsening of the clinical signs have been seen in certain patients after methylprednisolone therapy (Arsura et al., 1985; Munakata et al., 2002). We also observed the deterioration of clinical condition of the patient “A” after IV methylprednisolone. Since oral neostigmine therapy was initiated immediately after this, it was difficult to ascertain the effect of methylprednisolone on this patient. The key problem associated with neostigmine bromide is the development of adverse cholinergic reactions. In order to avoid that, neostigmine therapy could be initiated at lower dose rate in 4-5 divided doses.

REFERENCES


