Abstract

Objective – To review the human and veterinary literature on the pathophysiology of myasthenia gravis (MG) and describe treatment options for clinical use in people and animals.

Data Sources – Human and veterinary clinical reports, studies and reviews, textbooks, and recent research findings in MG from 1996 present, with a focus on treatment and patient management.

Human Data Synthesis – MG is a well-described condition in people with new research and treatment options available. Many of the newest therapeutic options available in veterinary medicine for MG are based on current strategies used in people with this condition. Seronegative MG is well described in people and provides insight to clinical cases encountered in veterinary medicine when the index of suspicion is high though serologic tests are negative.

Veterinary Data Synthesis – Previous studies in veterinary medicine focused on the use of acetylcholinesterase inhibitors as the main form of treatment in canine MG. Recent studies, mainly case series and case reports, emphasize the use of immunomodulatory treatments as an alternative for long-term treatment. However, there are no randomized, controlled studies on treatment with immunomodulatory therapy for MG in dogs available to assess the efficacy of this treatment strategy.

Conclusions – Although early recognition of clinical signs is most important in the outcome of patients with MG, further understanding the pathophysiology of MG may lead to earlier diagnosis and novel treatment strategies. The discovery of additional autoantibodies against striated muscle proteins in dogs, should enhance our understanding of diseases affecting the neuromuscular junction. In addition, clinical data for canine MG could be applied to other autoimmune disorders.

Keywords: immune-mediated disorders, megaesophagus, neuromuscular disorders

Introduction

Myasthenia gravis (MG) is a neuromuscular disorder caused by a reduction in the number of functional nicotinic acetylcholine receptors (AChR) on the postsynaptic membrane of the neuromuscular junction. The derivation of the terms ‘myasthenia gravis’ relates to ‘myo-’ or ‘my-’ signifying the involvement of muscle, ‘-asthenia’ relating to the major finding of generalized weakness, and ‘gravis’ for severity of the condition. MG exists as either a rare congenital disease or more commonly as an acquired autoimmune disease resulting in a deficiency of nicotinic AChR. Acetylcholine is essential for muscle contraction at the neuromuscular junction. The defect in transmission resulting from AChR loss results in focal or generalized muscle weakness and exhaustion.

AChRs are part of a superfamily of neurotransmitter-gated ion channels that contain 5 homologous subunits centered around a central ion channel. There are 2 subtypes of muscle AChRs, a fetal form found before innervation or after denervation with a subunit composition of χ1, γ, χ1, δ, and β1 and mature form found at endplates with subunit composition of χ1, ε, χ1, δ, and β1. Many individuals and dogs affected with MG have autoantibodies that bind to fetal AChRs and to a lesser extent, the mature AChRs. Congenital MG is an inherited group of disorders involving disruption of neuromuscular transmission by...
presynaptic, synaptic, or postsynaptic deficits. It can be caused by mutations in the AChR subunits; in people there have been greater than 60 different AChR mutations identified to be associated with congenital MG. Clinical signs of congenital MG in dogs first manifest between the ages of 6 and 12 weeks. Breeds recognized with the congenital form of MG include the Jack Russell Terrier, Springer Spaniel, Smooth Fox Terrier, and Gammel Dansk Honsehund. In a report from 2005, 3 miniature Smooth-haired Dachshund litters were diagnosed with MG at 8 weeks of age, and clinical signs resolved by 6 months of age.

The most common form of MG, acquired MG, is an immune-mediated disorder in which autoantibodies (typically of the immunoglobulin G class) target postsynaptic nicotinic AChRs of skeletal muscle. Familial autoimmune MG has been reported in Newfoundland and Great Dane dogs. Autoantibodies lead to a loss of AChRs at the neuromuscular junction and impair transmission of the action potential from nerve to muscle. In MG, most of the autoantibodies to AChR are directed against the main immunogenic region (MIR), a conformation-dependent region located at the extracellular tip of the α1-subunits. The structure of the MIR provides information on pathological mechanisms of how autoantibodies impair neuromuscular transmission in MG. The MIR is located at the top of the extracellular surface of AChRs and away from the ACh-binding site. It is angled away from the central axis of the AChR allowing antibodies to cross-link AChRs and induce antigenic modulation. High proportions of autoantibodies to the MIR have been identified in people and dogs with MG. Human, experimentally induced autoimmune myasthenia gravis, and canine studies demonstrate that greater than half of the autoantibodies in circulation are directed against the MIR.

In most cases, adequate acetylcholine is present. However, the receptors are deficient and therefore acetylcholine will not bind adequate numbers of AChRs to initiate an action potential. MG is an autoimmune T-lymphocyte-dependent disease with production of specific autoantibodies against muscle AChRs or other muscle proteins. A major factor in the pathogenesis of MG is activation of CD4+ T (helper) lymphocytes and interaction with B lymphocytes leading to autoantibody production. This differs from pure T-cell-mediated diseases such as multiple sclerosis where the T cells are mainly CD8+ (cytotoxic). The AChR-antibodies complexes formed result in immune-mediated destruction of the postsynaptic membrane and AChR loss. Under physiologic conditions regulatory T lymphocytes inhibit CD4+ T lymphocytes preventing the autoimmune process.

There is a safety margin for neuromuscular transmission defined as the ratio between the number of acetylcholine quanta released and the number of acetylcholine quanta required for depolarization of a muscle fiber. The safety margin is the amount of interference with the end-plate and transmission that can exist without failure of neuromuscular transmission. Normally there is an excess number of AChR, which produce an end-plate potential magnitude that is significantly larger than what is required for the muscle action potential threshold. Diseases that affect the neuromuscular junction, such as MG, reduce end-plate potential amplitude decreasing the safety factor. If the end-plate potential drops below the level required for the firing threshold, neuromuscular transmission is blocked. In MG, the amplitude of the muscle action potential with repetitive stimulation shows a progressive decline and a decreased safety factor.

There are 3 main mechanisms for loss of functional AChRs at the neuromuscular junction. The first involves complement-dependent lysis of the postsynaptic membrane caused by antibodies bound to AChRs and simplification of the postsynaptic membrane. Secondly, antibodies can cross-link AChRs on the surface of the membrane leading to increased internalization of AChRs, a decrease in the receptor half-life and decline of the total number of AChRs. In people, the half-life is reduced to 3 days from a normal of 10 days. Lastly, antibodies may directly inhibit AChR function. In all cases, there is reduction in neuromuscular transmission and decreased muscle contraction. Although acquired MG has been classically linked to a dysfunction in CD4+ T cells, the specific role of B cells needs to be further clarified. B cells are implicated in systemic autoimmune diseases by cytokine production, antigen presentation, and complement activation. Antigen-presenting B cells interact to activate T cells. This enhances antibody production and the immune response. Autoantibodies can activate Fc receptors on macrophages and dendritic cells leading to increased cytokine production, resulting in the activation of an acute inflammatory cascade and tissue damage.

Classifications of MG

Acquired MG has a bimodal age distribution in people and dogs. In people, 1 subset consists of women in their 20–30 years while the other group is men in their 60 and 70 years. Similarly, a bimodal age distribution has also been identified in dogs. MG is classified based on distribution and severity of clinical signs and is described as focal, generalized, or acute fulminating (Table 1). With focal MG, there is typically no historical or clinical evidence of thoracic or
pelvic limb muscle weakness. Instead, weakness is present in 1 or more muscle groups including the facial, esophageal, pharyngeal, and laryngeal muscles. A dog may present with megaesophagus alone or with involvement of the other muscles without evidence of generalized weakness.13 Focal MG occurs in 36–43% of all canine cases.11,28,29 In people 15% of all MG patients have the disease restricted to the eyes.26 Generalized forms of MG can manifest in a wide range of clinical presentations ranging from mild to severe weakness to megaesophagus. The pelvic limbs are affected with higher frequency than the thoracic limbs.30 Generalized MG occurs in 57–64% of all canine cases with 90% of them having megaesophagus.11,29

Fulminating MG presents with an acute onset and rapid development of clinical signs. Animals can develop tetraparesis, respiratory distress, aspiration pneumonia and may require mechanical ventilation for management. This form is associated with a higher mortality rate and carries a poorer prognosis due to severe respiratory muscle involvement.27 Fulminating MG occurs in <5% of affected dogs with generalized MG.11,29 Fulminating MG has also been documented in children but it is best described in adults; a recent study conducted in China with 159 adult humans with MG, identified that approximately 12% of cases had fulminating MG.31–33

A paraneoplastic form of MG can occur with thymomas as well as others neoplasms. There have been case reports of acquired MG in dogs with cholangiocellular carcinoma and CNS lymphoma.34,35 In people, 30–60% of thymomas are associated with MG.2 In dogs that have thymomas, 30–50% have been identified to have MG.36 The association between the presence of thymoma and MG is well characterized.36–39

### Clinical Presentation

Animals with MG can present with focal or generalized muscle weakness. Although muscle weakness will usually worsen with activity and improve with rest, cases with generalized weakness may present similarly to dogs with polyradiculoneuritis. Most dogs do not display the classical exercise intolerance presentation and instead only develop weakness. Some dogs will have ptosis of the upper eyelids, drooping of the lips, sialosis, regurgitation, or dysphagia.7 In contrast to people and cats, dogs are prone to megaesophagus because the canine esophagus contains more skeletal muscle than smooth muscle. In 1 study, evaluating 25 dogs that were seropositive for MG, 84% had megaesophagus.11 In another study of 1,154 cases of acquired MG in dogs, 90% of generalized MG cases had megaesophagus.29

The cases which require immediate critical care are those deemed to be in a myasthenic crisis. Animals may present in acute respiratory distress with dysphagia due to pharyngeal dysfunction, or regurgitation due to megaesophagus. If an animal has megaesophagus, there is a high risk of aspiration pneumonia and treatment may require oxygen supplementation, antibiotic therapy or mechanical ventilation for respiratory fatigue. In 1 report, up to 60% of canine deaths with MG were due to respiratory complications.40 In all cases of MG with megaesophagus or regurgitation, aspiration pneumonia is a potential risk and cause for hospitalization and intensive supportive care.

### Diagnosis

MG should be considered in any animal with acquired megaesophagus and dysphagia, or with weakness that worsens with exercise. A CBC, general biochemical profile, urinalysis, and thyroid panel are required to rule out other conditions resulting in or contributing to weakness. Conditions that should be considered include hypothyroidism, severe hypoglycemia secondary to islet cell neoplasia, diabetic neuropathy, and electrolyte abnormalities such as hypokalemia and hyperkalemia (eg, as seen with hypoaldosteronism). In addition thoracic radiographs will provide useful information in identifying megaesophagus, aspiration pneumonia, or a cranial mediastinal mass.40 Other differentials include other myopathies or neuropathies such as polyradiculoneuritis or polymyositis, endocrine disorders such as hypoaldosteronism or disorders of neuromuscular transmission such as tick paralysis, botulism, or organophosphate toxicity.27

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**Table 1:** Classifications of acquired myasthenia gravis

<table>
<thead>
<tr>
<th>Classifications</th>
<th>Clinical signs</th>
<th>Percent affected</th>
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<tbody>
<tr>
<td>Focal</td>
<td>Weakness present in 1 or more muscle groups</td>
<td>36–43</td>
</tr>
<tr>
<td></td>
<td>Typically no clinical evidence of thoracic or pelvic muscle weakness</td>
<td></td>
</tr>
<tr>
<td>Generalized</td>
<td>Wide range of clinical signs: mild to severe weakness or megaesophagus</td>
<td>57–64</td>
</tr>
<tr>
<td></td>
<td>Pelvic limbs affected more often than thoracic limbs</td>
<td></td>
</tr>
<tr>
<td>Fulminant</td>
<td>Acute onset and rapid development of clinical signs</td>
<td>&lt;5 of all generalized forms</td>
</tr>
<tr>
<td></td>
<td>Respiratory distress ± tetraparesis ± aspiration pneumonia</td>
<td></td>
</tr>
<tr>
<td>Paraneoplastic</td>
<td>May occur with thymoma</td>
<td>30–50 dogs with thymoma may develop MG</td>
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The neurological examination in MG dogs is often normal; however, in some cases it may be abnormal and cranial nerve abnormalities may be seen. The patellar tendon reflex is usually preserved but will fatigue with repeated stimulation. If an animal is severely weak, the clinician can easily misinterpret weakness for absent to decreased conscious proprioception. Even in the weakest animals, if weight is supported the animal should have no proprioception deficits. Some animals may have ventral flexion of the neck and cranial nerve deficits with difficulties in prehension, dysphagia, or re- gurgitation. Other signs may include facial muscle weakness, weak jaw tone, weak gag reflex, and laryngeal paralysis. Facial muscle weakness is usually manifested as a decremental blink reflex. Electromyography (EMG) findings are usually normal. Repetitive nerve-stimulation of the compound muscle action potential, may provide supportive evidence of MG. A decrease in amplitude of successive compound muscle action potentials is considered abnormal and may be supportive for MG. Single-fiber EMG is used to record motor unit action potentials in neuromuscular transmission and is based on the principle of jitter. Jitter is a measure of neuromuscular transmission that increases with the ratio between depolarization threshold and end-plate potential increases. In people with MG, 92–100% have abnormal jitter values. Although single-fiber EMG is a very sensitive test for supporting a disorder of neuromuscular transmission in people, both EMG and repetitive nerve-stimulation tests require general anesthesia in dogs, which can present serious risks, expense and compromise to the patient. It is also technically very difficult to perform and not widely available.

The edrophonium chloride response test is a clinical pharmacological test for a rapid presumptive diagnosis of MG using an ultra-short acting anticholinesterase agent, edrophonium chloride. A positive response is defined as a temporary increase in muscle strength. This test is not specific or sensitive and has limitations, but a dramatic positive response is suggestive of MG. The dose of edrophonium chloride described for this test is 0.1–0.2 mg/kg IV in dogs. False-positive and false-negative results are possible and therefore the results should be interpreted with some caution. Some myasthenic dogs are not edrophonium responsive while other neuromuscular disorders may yield a positive response. No improvement in muscle strength does not exclude a diagnosis of MG. Dogs with generalized MG often have a positive response to the edrophonium response test while fulminant MG dogs typically do not. Fulminant dogs may not have enough AChRs available to elicit a noticeable response. Documentation of a positive response to the edrophonium chloride test with focal MG is more problematic. However, the palpebral reflex may return in cases of focal MG, so the reflex should be tested after edrophonium chloride is administered.

The gold standard for the diagnosis of MG in both people and dogs is the documentation of autoantibodies against muscle AChRs by immunoprecipitation radioimmunoassay (RIA). This assay is specific, sensitive, and documents an autoimmune response against muscle AChRs. A ChR antibody titer > 0.6 nmol/L is diagnostic for MG in dogs. Despite its usefulness in the diagnosis of acquired MG, the test does have its limitations. It is not a predictor of the degree of weakness or severity of the disease between animals. However, in the absence of immunosuppression, there is a clear relationship between the AChR antibody titer and the clinical course of disease in an individual animal. Immunosuppressant drugs will decrease autoantibody titers. In a study, it was estimated that nearly 10–15% of all dogs with generalized MG are seronegative. However, this estimation was extrapolated from the human MG literature and not from the above mentioned clinical study. In another study, 2% of dogs with generalized clinical signs consistent with MG and a positive edrophonium response test were seronegative. Single-fiber EMG is used to diagnose a disorder of neuromuscular transmission, and in people, for a presumptive diagnosis of seronegative MG (SNMG).

In comparison, an older study dating back to 1974, reported 85% of human myasthenic patients had measurable serum antibodies to human muscle AChRs. In a recent human review, 15% of patients without AChR antibodies had antibodies directed against other muscle proteins such as muscle-specific receptor tyrosine kinase (MuSK). Autoantibodies against skeletal proteins other than AChRs such as titin and ryanodine receptor (RyR) are documented in human and canine MG patients with a thymoma. Interestingly, the combination of ACR and RyR antibodies increases the severity of myasthenia.

Nonimmune Considerations and Therapies

In all cases of MG, there are major considerations for clinicians beyond drug therapy including oxygen and respiratory therapy, temperature control, and potential surgical treatments. In people, 10–20% of MG patients require mechanical ventilation and mortality due to complications are estimated at 11–14%. Respiratory failure or fatigue may occur if an animal presents in a myasthenic crisis. An animal may similarly present in respiratory distress secondary to treatment with excessive doses of cholinesterase inhibitors. It is therefore important to differentiate a myasthenic crisis from a
cholinergic crisis in order to initiate appropriate therapy.\textsuperscript{50} Both types of patients present with respiratory difficulty or failure and focal or generalized muscle weakness which can make it challenging to make the diagnosis. It may be necessary to intubate dogs before differentiating the 2 and provide manual ventilation and oxygen support.\textsuperscript{50} A mode of differentiating between myasthenia versus cholinergic crisis is with an endrophonium response test. If the respiratory distress is due to a cholinergic crisis, the endrophonium test will result in muscle fasciculations, miosis, and worsening of respiration. If an animal’s respiratory failure improves following the endrophonium test, then it likely is an animal in a myasthenic crisis.\textsuperscript{50} It is important to note, however, this is not a specific or sensitive test for MG.

In human medicine, patients have the advantage of avoiding full ventilation by the use of bi-level positive airway pressure, a type of respiratory support that provides oxygen and pressure via a mask and tubing. The machine supplies pressure to the lungs when the patient breathes; this holds open the air sacs and supplements oxygen to the alveoli. In a retrospective study of human patients, an initial measurement of $pCO_2 > 45$ mm Hg is associated with bi-level positive airway pressure failure. This lends credence to the concept that mechanical ventilation is needed for these patients to survive.\textsuperscript{50} In people who were ventilated for myasthenic crisis, predictors of respiratory failure included presence of atelectasis, low blood pH, and forced vital capacity at extubation. The overall goal of ventilation in these patients is to avoid atelectasis.\textsuperscript{51} Respiratory support is particularly important for treatment of aspiration pneumonia in myasthenic dogs.

Mild hypothermia causes decreased muscle temperature which improves synaptic transmission because neuromuscular transmission is temperature-dependent.\textsuperscript{51} A cooler body temperature also appears to improve pulmonary function and therefore, maintaining cooler body temperature is being advocated in the management of human myasthenia patients.\textsuperscript{51} This has not been evaluated in dogs. Limited access to special equipment that allows effective control of core body temperature in veterinary medicine is a major disadvantage compared with human medicine.

**Seropositive and SNMG**

In MG, antibodies against striated muscle proteins such as myosin, actin, $\alpha$-actinin, or titin can occur in addition to AChR antibodies. These antibodies are called anti-striational antibodies that occur in combination with AChR antibodies in cases of thymoma and MG in people and dogs.\textsuperscript{52} A well-characterized form of AChR antibody-negative MG in people is associated with autoantibodies against the enzyme, MuSK. This enzyme is located near the AChR. It is activated by agrin, a proteoglycan that binds to MuSK in the neuromuscular junction when released from motor neurons.\textsuperscript{53} MuSK induces acetylcholine clustering at the postsynaptic membrane. MuSK autoantibodies are detected in up to 70% of generalized acetylcholine-receptor-antibody-negative MG patients.\textsuperscript{54} The endrophonium response test has been positive in 50% of MuSK-Ab positive cases in 2 human studies.\textsuperscript{55,56} Some differences in presentation and severity are appreciated between MuSK-Ab positive MG and AChR-Ab positive MG. MuSK-Ab positive cases tend to have a more ocular distribution and therefore higher percentage of focal MG.\textsuperscript{57} Additionally, thymomas are rare in MuSK-Ab positive cases.\textsuperscript{50} One seronegative myasthenic dog has been documented with autoantibodies against MuSK.\textsuperscript{a}

Myasthenic humans that do not have antibodies to either AChR or MuSK are termed seronegative MG. In a study of SNMG, AChR antibodies (mainly IgG1 subclass) to a clustering protein called rapsyn were detected in 66% (25/38) of MG patients that were seronegative for AChR binding antibodies.\textsuperscript{58}

Another muscle protein that may be associated with AChR seropositive MG is the RyR. Antibodies against RyR have been documented in human and canine AChR seropositive MG cases that are particularly severe.\textsuperscript{48} RyR is a calcium release channel in striated muscle that is necessary for muscle contraction.\textsuperscript{50} Deficiency in both neuromuscular transmission and in muscle contraction can worsen the severity of muscle weakness. Antibodies against the RyR in conjunction with AChR\textsuperscript{51} autoantibodies is associated with a severe form of thymoma related MG in people and dogs.\textsuperscript{48,52}

The criteria for a diagnosis of SNMG in dogs include compatible clinical signs, a positive edrophonium response test, electrophysiologic findings such as decremental response to repetitive motor nerve stimulation, normal limb muscle strength following anticholinesterase therapy, and at least 2 seronegative AChR antibody titers.\textsuperscript{26} Explanations for SNMG focus on antibodies to muscle proteins other than AChRs. In addition, antibodies can be directed against the $\alpha$-bungarotoxin binding site, which would not be detected in the standard RIA.\textsuperscript{28} In this assay, the antibodies bind to the bugarotoxin binding site blocking the $\alpha$-bungarotoxin from binding.\textsuperscript{28} The AChR antibodies bind as a competitive inhibitor to the $\alpha$-bungarotoxin.\textsuperscript{3} In 20 SNMG dogs with a positive edrophonium response test, 5 dogs had AChR antibody detected within the muscle by biochemical quantification. These dogs had a low muscle AChR content and antibody bound to muscle AChR.\textsuperscript{59}
There is evidence suggesting that AChR antibody seropositive MG may differ in mechanism from MuSK MG. AChR and MuSK antibody-seropositive myasthenias differ in IgG subclass response. In people, AChR antibody seropositive MG has complement fixing IgG1 and IgG3 subclasses predominantly. In contrast, MuSK antibody positive MG has complement fixing IgG4 subclass. Protein antigens typically provoke IgG1 and IgG3 responses, whereas chronic antigen stimulation is more characteristic of IgG4 subclass. The IgG4 tends to involve autoimmune diseases due to this chronic nature. Lastly, IgG2 subclass predominates in response to carbohydrate antigens.

**Treatment**

There are many therapeutic options for acquired MG (Table 2); treatment therefore needs to be individualized for each patient’s needs. The overall goal is to improve muscle strength and minimize the adverse effects of medications until the disease goes into remission. A critical difference between human and canine MG is that, in the absence of immunosuppression, the natural course of canine MG is for spontaneous immune remission. A 2001 study found that 47/53 dogs with MG that were seropositive for AChR antibodies and were treated with anticholinesterase therapy alone went into remission within an average of 6.4 months. This study was initiated to determine the natural course of the disease in the absence of immunosuppression and included only those dogs that survived to remission and not dogs that died of aspiration pneumonia early in the course of the disease. In general, the treatment plan may need to be altered based on response and severity of the disease. For mild disease, anticholinesterase agents and altered feeding procedures may be sufficient. For more severe disease, early use of immune suppressants or immunomodulators (a substance that has some effect on the immune system) may be required.

**Acetylcholinesterase Inhibitors**

Acetylcholinesterase (AChE) inhibitors have been the foundation of therapy for acquired MG and are often the first line of therapy. The mechanism of action of AChE inhibitors is to inhibit hydrolysis of acetylcholine at the neuromuscular junction prolonging the action of acetylcholine. Cholinergic and muscarinic side effects may occur with cholinesterase inhibitors, thus dosages must be titrated for optimal benefit with minimal adverse effects. Animals can develop a cholinergic crisis and present with weakness, abdominal pain, hypersalivation, and diarrhea. A possible disadvantage of AChE inhibitors is that it is only symptomatic treatment, and does not address the autoimmune pathogenesis of the disease. However, unlike in people, spontaneous remissions occur in dogs and symptomatic treatment may be adequate until remission occurs. An additional benefit is that dogs that go into remission on AChE drugs alone may have a better chance of staying in remission than if immunosuppression was used. Pyridostigmine bromide and neostigmine bromide are the most commonly used AChE inhibitors. Pyridostigmine bromide is preferred in most clinical situations because of its longer duration of action and fewer side effects.

Both pyridostigmine bromide and neostigmine bromide inhibit the hydrolysis of ACh by directly competing with ACh for attachment to AChE. Pyridostigmine bromide is better tolerated than neostigmine bromide with fewer gastrointestinal adverse effects. The dosage is 0.5–3.0 mg/kg administered orally twice to thrice daily; it is available in syrup, tablet, and timed-release forms. Pyridostigmine bromide may also be given as a constant rate infusion at 0.01–0.03 mg/kg/h IV. Some common adverse effects (1–10% in people) include muscarinic symptoms; these include increased gastrointestinal motility, diarrhea, and abdominal pain. Adverse effects occur because muscarinic receptors located on the exocrine glands increase gastric acid secretion, salivation, and lacrimation. Bradycardia can be seen due to excessive vagal activity. Side effects seen in people are similar in dogs.

In a rat model studying the long-term effect of pyridostigmine bromide, degeneration of the postsynaptic folds was noticed in the endplates on red muscle fibers. In human MG, chronic administration of AChE inhibitors are not thought to damage the neuromuscular junction. In a study of 117 myasthenic human patients, 51% were treated with pyridostigmine bromide. Of the group of people treated with pyridostigmine bromide, 64% suffered from daily muscarinic adverse effects with gastrointestinal effects reported most commonly. Thirty-six percent of these patients had additional adverse effects such as muscle fasciculation and fatigue. Immunosuppressive medications are often the first choice in older human patients with MG to avoid these adverse effects.

**Corticosteroids**

Corticosteroids are commonly used to treat many autoimmune diseases. They are often used in the management of human MG and, therefore, should be considered in canine MG patients. Corticosteroids are listed as a Class II evidence (controlled trial without randomization or randomized trial with small patient data).
number) for use in generalized MG in people. They are used as a first-line therapy for only a short period in people. Their precise mechanism of action is unknown. However, they are believed to bind to intracellular receptors that regulate gene expression, thereby down regulating immune function. They also decrease neutrophil migration, inhibit macrophages and inhibit the release of inflammatory cytokines from macrophages. Corticosteroids are thought to inhibit T-cell activation by inhibiting the activation process in the cell nucleus and inhibiting antigen processing. The dosage used is anti-inflammatory (0.5–1.0 mg/kg/d orally) and reduced in an appropriate tapering schedule. In mild or moderately affected human patients, low-dose prednisone therapy has been recommended (0.5 mg/kg every other day) in order to minimize adverse effects. Immunosuppressant drugs may be of value early in the course of MG in dogs when clinical signs may be most severe and difficult to control.

**Azathioprine**

Azathioprine is a purine analogue that acts on proliferating lymphocytes and induces both B-cell and T-cell lymphopenia. It is used most often with corticosteroids to reduce the amount of steroids required. It is listed as a Class I evidence (randomized-controlled trials are available) recommendation for MG in people and used as a first-line therapy for long-term use. Azathioprine gets converted to 6-mercaptopurine in the liver. It works by decreasing lymphocyte proliferation and is specific to T lymphocytes. The dosage is 1–2 mg/kg orally once a day or every other day. A CBC should be obtained 2 weeks after initiating azathioprine therapy, then monthly thereafter. Azathioprine is tolerated very well by most people and dogs although adverse effects such as liver failure and bone marrow suppression are possible. Complete immune response to azathioprine may take up to 6 weeks but in many cases clinical response is seen within 2 weeks. There are also drug interactions that are possible with azathioprine. For example, allopurinol impairs the conversion of azathioprine to 6-thiouric acid interfering with metabolism of the drug. Although not as readily available, azathioprine is available in an intravenous form as 100 mg/vial (20 mL). In people with MG, azathioprine is the most widely used long-term immunosuppressant apart from corticosteroids. Azathioprine may also increase the risk of central nervous system lymphoma in people although the link is uncertain. The association was found in human myasthenic patients that were on azathioprine between 6 and 12 years; this may not be a factor in veterinary medicine given the life span of companion animals and the high rate of immune remission.
## Table 2: Treatment for myasthenia gravis in veterinary medicine

<table>
<thead>
<tr>
<th>Drug</th>
<th>Class of drug</th>
<th>Dose (mg/kg)</th>
<th>Use/indications</th>
</tr>
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<tbody>
<tr>
<td>Pyridostigmine bromide (Mestinon)</td>
<td>Cholinesterase inhibitor</td>
<td>1–3 mg/kg, PO, q 8–12 h, 0.01–0.03 mg/kg/h, CRI, IV</td>
<td>Uses Myasthenia gravis, Adverse effects Increased GI motility, Diarrhea, Abdominal pain, salivation, Lacrimation, Bradycardia</td>
</tr>
<tr>
<td>Neostigmine bromide (Prostigmin)</td>
<td>Acetylcholinesterase inhibitor Parasympathomimetic</td>
<td>2 mg/kg/d, PO</td>
<td>Uses Myasthenia gravis, Initiate peristalsis, Empty bladder, Stimulate skeletal, Muscle contractions, Treat massive ivermectin overdose, Adverse effects Cholinergic signs</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>Corticosteroids</td>
<td>0.5–1.0 mg/kg/d, PO Followed by taper, 0.1 mg/kg, IV, Dex SP</td>
<td>Uses Anti-inflammatory, Immunosuppression, Adverse effects Polyuria, polydipsia, Panting, diabetes, Obesity, muscle wasting, GI ulcers, Infection risk</td>
</tr>
<tr>
<td>Azathioprine (Imuran)</td>
<td>Antimetabolite Purine analog</td>
<td>1–2 mg/kg, PO, q 24–48 h</td>
<td>Uses Immunosuppressant, Adverse effects Liver failure, Bone marrow suppression, Pancreatitis, Hepatotoxicity</td>
</tr>
<tr>
<td>Cyclosporine A Microemulsified form (cyclosporine modified)</td>
<td>Calcineurin inhibitor</td>
<td>3–6 mg/kg, PO or IV, q 12 h</td>
<td>Uses IMHA, ITP, KCS, Perianal fistulas, Atopic dermatitis, Pemphigus foliaceous, GME, IBD, Adverse effects Dose dependent, Nephrotoxicity, Hepatic disorders, Hypertension, Gingival hyperplasia, Weight loss, Allergic reactions</td>
</tr>
<tr>
<td>Leflunomide</td>
<td>Antimetabolite Isoxazol derivative</td>
<td>1.5–5 mg/kg, PO, q 24 h, Trough level in 30 days</td>
<td>Uses Steroid-resistant IMHA, ITP, GME, SLE, Adverse effects GI toxicity, anorexia, Vomiting, mild anemia, Bone marrow Suppression</td>
</tr>
<tr>
<td>Mycophenolate mofetil</td>
<td>Inhibitor of purine synthesis</td>
<td>7–15 mg/kg, PO, q 12 h, IV dose: 15 mg/kg</td>
<td>Uses Rheumatoid arthritis, Myasthenia gravis</td>
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another study involving 41 human MG patients, azathioprine (plus prednisolone for the first month) had no benefit over prednisolone alone. Adverse effects of azathioprine reported in humans include acute idiosyncratic reaction, fever, skin reactions, gastrointestinal symptoms, leukopenia, and elevated liver enzymes. Ten percent of people have adverse effects such as influenza-like illness, bone marrow suppression, and liver toxicity from its use.

In a veterinary study of 5 dogs with megaesophagus treated with azathioprine for acquired MG, 4/5 dogs showed a therapeutic sequential decline in serum AChR antibody concentration while receiving azathioprine therapy. It is important to note that 4/5 dogs were receiving pyridostigmine therapy before implementing azathioprine therapy and 1 dog was treated temporarily with an immunosuppressive dose of prednisone.

**Cyclosporine A**

Cyclosporine A has been frequently used to treat autoimmune diseases of animals. It is listed as a Class I evidence recommendation for use in generalized MG in people, particularly for those who cannot tolerate or are nonresponsive to azathioprine, methotrexate, MMF, prednisone, or tacrolimus. Cyclosporine inhibits T-cell activation and prevents synthesis of several cytokines in particular, interleukin 2 (IL-2). It blocks calcineurin-mediated cytokine signaling. Cyclosporine binds to a cyclophilin, immunophilin in the cytoplasm, binding and blocking the function of calcineurin, an enzyme necessary for T-cell activation. The dosage is 6–12 mg/kg orally per day divided into 2 doses and 3–6 mg/kg twice a day IV. Cyclosporine was the first effective drug for human myasthenic patients that was evaluated in a prospective, double-blind placebo-controlled trial. It is as effective as azathioprine but acts more rapidly with fewer adverse effects. Cyclosporine does not cause myelosuppression or suppression of innate immunity. The adverse effects of cyclosporine are dose-dependent nephrotoxicity, hepatic disorders, hypertension, gingival hyperplasia, weight loss, and allergic reactions in people. Generic cyclosporine is available but still costly and blood concentration monitoring is required for effective use due to a narrow therapeutic index. In people, it has been demonstrated that measuring concentrations of cyclosporine 2 hours after administration and before administration is useful to estimate efficacy and safety.

A case study of 2 dogs treated with cyclosporine showed promise for its use in MG. Both dogs failed therapy with pyridostigmine bromide and prednisone alone. Cyclosporine was effective in achieving clinical remission in these 2 cases. One of the dogs that went into clinical remission reverted to seropositivity and clinical signs of MG when the immunosuppressive therapy was stopped. It is difficult to ascertain whether or not the other dog in the study went into clinical or immune remission because the dog was continued on cyclosporine throughout the study.

In people a documented randomized, blinded, placebo-controlled trial demonstrated that cyclosporine was more effective than placebo in improving clinical assessment and lowering serum AChR concentrations. The maximum effects of cyclosporine usually take several months to achieve.

**MMF**

MMF is an inhibitor of purine synthesis in both T and B lymphocytes. It is 97% protein bound. MMF acts on DNA metabolism by noncompetitive, reversible inhibi-
tion of inosine monophosphate dehydrogenase, which is needed for synthesis of guanosine triphosphate. Purines are synthesized via 2 pathways, the de novo pathway and the salvage pathway. Lymphocytes will only use the de novo pathway when synthesizing necessary purines; this pathway is competitively regulated. It is down regulated by adenosine nucleotides and up regulated by guanosine nucleotides. MMF blocks the synthesis of guanosine, thereby enhancing the synthesis of adenosine. Both result in inhibition of purine synthesis in the lymphocytes. Other effects of MMF include limiting production of nitric oxide, reducing secretion of tumor necrosis factor α, and reducing proliferation of T and B lymphocytes. MMF is rapidly absorbed orally and can be administered IV in regurgitating patients. Intravenous dosages have been reported as 15 mg/kg diluted in 500 mL of 0.45% saline and 2.5% dextrose over 4 hours or 16 mg/kg IV over 2 hours. The oral dosage of MMF in dogs is 7–15 mg/kg twice daily. Adverse effects are dose-dependent and can include bone marrow suppression, gastrointestinal upset, nausea, peripheral edema, infection, development of lymphoma, sepsis, hypertension, tremors, chronic heart failure, and primary CNS lymphoma.

A recent case series of 3 dogs with severe generalized MG treated with MMF demonstrated clinical remission in 48 hours with no adverse effects. All 3 animals were seropositive for AChR antibodies; this was the first report in dogs successfully demonstrating the use of MMF in treatment of severe generalized MG. In another case report, a 10-year-old male castrated Springer Spaniel diagnosed with focal MG (seropositive for AChR antibodies) initially improved with azathioprine but deteriorated shortly after. MMF was orally instituted in addition to azathioprine resulting in dramatic improvement. However, in a recent retrospective cohort study of 27 dogs with serologically confirmed acquired MG that were treated with a combination of MMF and pyridostigmine compared with those treated with pyridostigmine alone did not show any beneficial therapeutic effect of MMF over the long term.

There have been a number of studies on the use of MMF in human medicine. A retrospective study of MMF in neuromuscular disease included 38 patients (32 with MG). In this study, MMF was used as a corticosteroid-sparing agent or as additional autoimmune therapy. Fifteen of the myasthenic patients had undergone a thymectomy and 4 received mycophenolate as a sole means of therapy. Sixty-three percent (24 patients) of patients benefited from MMF; this was defined as improvement in functional status or a corticosteroid-sparing effect. MMF allowed patients to reduce their dose of steroids in 50% of the cases. Patients who had been taking MMF for a longer period benefited the most. Patients who did not respond were treated for a shorter time period and had longer duration of disease. A human case report involved a woman with MG that was treated with MMF and prednisone for 6 years and then developed Epstein-Barr virus positive T-cell lymphoproliferative disorder. This raises questions of possible serious adverse effects of this drug in people. There are 2 recent randomized, double-blinded placebo-controlled prospective studies that did not show benefit in treatment of acquired MG in people with MMF over prednisone.

### Leflunomide

Leflunomide is an isoxazol derivative with novel immunomodulating properties. It is used in treatment for rheumatoid arthritis, systemic lupus erythematosus, Wegener’s granulomatosis, Crohn’s disease, and solid tumors in people. Leflunomide’s immunomodulatory activity is credited to its primary metabolite, A77 1726, that inhibits T- and B-cell proliferation, suppresses immunoglobulin production, and interferes with cell adhesion. In a study of dogs with immune-mediated disorders (eg, immune-mediated thrombocytopenia, immune-mediated hemolytic anemia [IMHA] or inflammatory diseases [eg, multifocal nonsuppurative encephalitis/meningomyelitis, systemic histiocytosis]) unresponsive to conventional therapy or subjected to glucocorticosteroid toxicity, leflunomide was able to control or treat with few harmful effects. In a study of 2 groups of rats immunized with purified AChRs, the group that did not receive leflunomide developed experimental autoimmune MG while the rats treated with leflunomide did not. The dosage is 1.5–5.5 mg/kg given orally once a day in dogs. Adverse effects include decreased appetite, lethargy, gastrointestinal upset, and bone marrow suppression.

### Cyclophosphamide

Cyclophosphamide is a strong alkylating agent that acts on DNA replication, RNA transcription and replication, to disrupt nucleic acid function and inhibit cell proliferation. In people, it is used in MG patients refractory to other treatments. Studies have shown that intravenous pulses of cyclophosphamide allowed reduction of systemic corticosteroid use without muscle strength deterioration or other adverse effects. In the Cochrane Library review 2009, cyclophosphamide was reported to be statistically more efficacious than placebo at 12 months in glucocorticosteroid-dependent patients (n = 21 participants). There is no well documented use of cyclophosphamide in myasthenic patients in veterinary medicine. The adverse effects include bone marrow
suppression, infections, bladder toxicity, and in people, dose-related neoplasms. A CBC should be performed 7–10 days after initiating treatment with cyclophosphamide.

**Tacrolimus**

Tacrolimus is a macrolide compound isolated from Streptomyces tsukubanensis. It acts on T helper cells to suppress production of cytokines, specifically IL-2. It is used to prevent organ rejection by human transplant patients. A human study of MG patients treated with tacrolimus for 16 weeks found that 47% showed improvement and reduction of AChR antibody titers and IL-2 production. Another study of 12 patients given tacrolimus for 2 years found 67% reported improvement in clinical signs. In a retrospective clinical review of 212 myasthenic patients, low-dose tacrolimus was documented as an effective approach to treatment in either reducing the dose of immunosuppressant and/or after unsuccessful thymectomy. There are no reported cases of thymectomy in canine MG.

**Etanercept**

Etanercept is a soluble recombinant tumor necrosis factor α receptor Fc, a protein found on cells that binds to antibodies. TNF-α is a proinflammatory cytokine that plays a role in many autoimmune diseases. In a pilot-open label clinical trial of etanercept in a group of corticosteroid-dependent human MG patients (n = 11), 1 patient had severe clinical worsening presumably due to an etanercept-induced rise in circulating TNF-α. This study suggests that myasthenic human patients may have more TNF-α receptors on their T cells. Etanercept is used in the treatment of rheumatoid arthritis, ankylosing spondylitis, and psoriasis in people. It has limited use in human medicine but may have potential use in refractory cases of MG. There are no reported studies of etanercept use in veterinary medicine.

**Rituximab**

Rituximab is a chimeric IgGk monoclonal antibody that targets human CD20 antigen. CD20 is expressed on the surface of normal and malignant B cells. Rituximab is used in treatment of relapsing/refractory CD20-positive-low grade non-Hodgkin’s lymphoma and autoimmune neuromuscular diseases such as IMHA, rheumatoid arthritis, and systemic lupus erythematosus. Rituximab is a monoclonal anti-CD20 B-cell antibody that targets B-lymphocyte antigen CD 20, which is necessary for B cell activation, differentiation, and growth. It depletes B cells by complement-mediated cytotoxicity and inducing apoptosis. Adverse effects include fever, chills, nausea, vomiting, bronchospasms, neutropenia, and increased risk of infection in people.

In a prospective study of 6 adults with refractory MG (5 were AChR seronegative), rituximab was considered a successful treatment. It is B-cell selective and is tolerated well with minimal adverse effects in people. There are no current recommendations for its use in human MG as further evaluation and studies need to be explored. Rituximab use has not been reported in veterinary medicine. Rituximab is specific to human B lymphocytes and therefore does not bind to canine B cells. In the future, antibodies that target canine B cell antigens such as CD21 may be considered for targeted therapy of autoantibody-mediated autoimmune diseases in this species.

**Thymectomy**

Thymic hyperplasia is relatively common in people with autoimmune MG, and although thymectomy is commonly performed, there are no prospective, randomized-controlled clinical trials documenting the value of thymectomy in treating human MG patients. The incidence of thymic hyperplasia in canine MG is not known. Thymomas can be associated with a myasthenic paraneoplastic syndrome in both people and dogs. Thymectomy is recommended in people with malignant thymoma associated MG. It is suggested that surgical excision of the thymus and mass removes the antigenic stimulus for AChR antibody production. It is not recommended in patients with antibodies to MuSK. MG occurs in 15–30% of people with thymomas, and of these patients, there is a 10% chance of having an associated thymoma. In human MG patients that do not have a thymoma, 70% of patients have thymic lymphofollicular hyperplasia. In dogs that have thymomas, 30–50% have MG. A 1994 study, involving dogs with thymomas demonstrated that dogs have a shorter survival time following thymectomy if they had megaesophagus as well. In a retrospective study of 1,154 cases of confirmed acquired MG dogs, 3.4% had an anterior mediastinal mass. Canine MG may be associated with a more severe form of MG in cases with thymomas. The presence of titin and RyR antibodies in addition to AChR antibody may result in more severe disease.

**Human IV Immunoglobulin**

The complement system is part of the immune system that carries both innate and acquired mechanisms and functions through a series of pathways involving covalent binding to proteins. Antibodies, such as those
directed at the AChR, can trigger the complement system. Human intravenous immunoglobulin (IVIG) involves interaction with inhibitory Fc receptors on phagocytic and antigen-presenting cells by enhancing expression of the inhibitory IgG Fc receptors. It directly neutralizes the blocking effects of AChR-antibodies targeting co-stimulatory molecules, cytokines, chemokines, B-cells, T-cells, Fc receptors, and the complement system. The anti-inflammatory property of IgG is due to sialylation of the N-linked glycan of the IgG Fc fragment. IVIG impedes stimulatory molecules and suppresses antibody production; it interferes with activating complement and formation of membrane attack complex. IVIG interrupts the complement activation pathway at the C3 stage. In a rat model of experimentally induced MG, intravenous immunoglobulin led to significant improvement in clinical signs with a decrease in AChR antibody titers.

IVIG is better tolerated than plasmapheresis in people with fewer adverse effects. It is manufactured through fractionation of blood from numerous human blood donors. It has recently been approved by the Food and Drug Administration for the treatment of chronic inflammatory demyelinating polyneuropathy in people. In dogs, IVIG has been used to treat autoimmune diseases such as IMHA, immune-mediated thrombocytopenia, and pemphigus foliaceous. There is documented use of IVIG in 2 dogs with MG using a dosage of 0.5 g/kg IV over 6 hours. To date, there are not enough data in veterinary medicine to comment on any benefits of IVIG in canine MG. IVIG must be used with caution in repeated doses due to anaphylaxis. Common adverse effects in people include headaches, myalgia, fever, chills, and nausea. To date in human MG, studies show no benefit of IVIG over plasmapheresis but both can improve clinical symptoms and may be considered in fulminating cases of MG.

**Plasmapheresis**

Plasmapheresis has been used in the treatment of autoimmune neurologic diseases in people such as MG, multiple sclerosis, Guillain-Barré syndrome, paraproteinemic neuropathy, chronic inflammatory demyelinating polyneuropathy, and acute disseminated encephalomyelitis. Plasmapheresis can occur via 2 techniques. The plasma can be separated by centrifuge or membrane filtration. The goal is to remove pathogenic antibodies and cytokines. This technique is not routinely used in veterinary medicine due to cost, equipment, and specialty training involved. A recent report documented the first veterinary case that used the membrane filtration technique for therapeutic plasmapheresis; this case was for IMHA. This case provides additional support for the use of therapeutic plasmapheresis in cases of autoimmune diseases. In particular, the most severe cases of MG, such as fulminant MG, may benefit from therapeutic plasmapheresis. Plasmapheresis reduces AChR antibody titers by 70% in this single canine case. Plasmapheresis, however, provides only a temporary improvement in clinical signs and must be repeated. It may be of use in severe refractory cases early in the course of the disease.

**Other Considerations**

Early and correct diagnosis of MG is of utmost importance in obtaining an optimal response to treatment of clinical signs and preventing life-threatening complications. There are many considerations in the management and care of dogs with MG including anesthesia, ways to avoid aspiration pneumonia, and concurrent diseases. If an animal with MG must undergo anesthesia for a life-threatening condition, there needs to be a detailed preanesthetic and postanesthetic evaluation. Preoperatively, an animal that is poorly controlled may benefit from other modalities such as plasmapheresis or IVIG therapy. An animal may require mechanical ventilation during recovery or if it has significant aspiration pneumonia. There is a long list of drugs that may worsen MG by blocking action at the neuromuscular junction. Some drugs to avoid include aminoglycosides, ampicillin, lidocaine, propranolol, and other β blockers, quinidine, procainamide, penicillamine, magnesium, and contrast agents. In people with MG, there is a small population (1–2%) that develop MG after being treated with penicillamine for rheumatoid arthritis. Stopping penicillamine therapy results in MG remission. As in most autoimmune disorders, infection
can be a trigger and an aggravating factor of MG. Spaying myasthenic female dogs is beneficial in preventing hormonal effects that can exacerbate MG.

Many myasthenic dogs have megaesophagus and therefore require altered feeding procedures in order to minimize aspiration pneumonia. The normal dog stands as a quadruped placing the esophagus in a horizontal position during ingestion of food. In order to reduce the risk of aspiration pneumonia, a specially designed feeding chair (see Figure 1) can be used. This feeding chair was originally designed by an owner of a dog with megaesophagus. The dog sits in an upright position for feedings as if in a high chair. Ideally the dog sits in the upright position for at least 10–15 minutes after eating to allow the food to move into the stomach. More information is available on the Megaesophagus Support Group website. Alternatively, owners can feed their pet in an elevated position on stairs or on a raised platform (Figure 1).

Vaccines for the Future?

The potential treatment modality of therapeutic vaccination may be a possibility 1 day for canine MG and other spontaneous autoimmune diseases. In the experimentally induced autoimmune myasthenia gravis model, residues 361–76 reactive B cells and 100–116 reactive T cells are the MIR for the nicotinic AChR. Vaccines were designed by developing peptides that bind to these target areas acting like antigen receptor mimics. The goal is for the anti-antigen receptor mimics Ab/Ag receptor complex to interfere with the T and B cell function and reduce concentrations of AChR autoantibodies. A prospectively studied group of 10 AChR Ab-positive myasthenic dogs with focal MG immunized with the T and B cell vaccines showed clinical remission rates that were increased compared with a historical control group of dogs that were not vaccinated. In addition, the AChR Ab concentrations declined greater in the vaccinated group versus the nonvaccinated group. Unfortunately, this study was not adequately controlled with an irrelevant vaccination group. Although promising, there are limitations to this study including sample size and control groups. Further extensive studies need to be investigated before a true conclusion on the efficacy and use of vaccines can be made.

Conclusions

Recent research has advanced our understanding of the neuromuscular junction and its various proteins. This has allowed for better diagnostic techniques to identify MG patients sooner and to rapidly provide supportive, functional and in some cases targeted immunomodulating therapies. The conventional method of treating MG with AChE inhibitors is still a viable treatment option, although immunosuppressant therapy may be required for severe cases early in the course of the disease. It is clear that carefully controlled clinical trials are necessary to determine the most effective mode of therapy. As in human MG, no specific treatment will be applicable to all cases of MG and treatment protocols will need to be tailored to the severity and response of each individual patient. There is a substantial amount of research that remains to be done to better understand the immune system and the role of immune suppressants in managing a myasthenic crisis.

Footnotes

a Shelton GD. Unpublished data, 2011.
b Mestinon, Valeant Pharmaceutical International, Aliso Viejo, CA.
c Prostigmin, ICN Pharmaceuticals Inc, Costa Mesa, CA.
d Available at: https://vetneuromuscular.ucsd.edu

References


Figure 1: This picture depicts a dog with myasthenia gravis in a specially designed feeding chair.


