Mycophenolate mofetil treatment in dogs with serologically diagnosed acquired myasthenia gravis: 27 cases (1999–2008)

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Objective—To compare clinical outcome in dogs with serologically diagnosed acquired myasthenia gravis (MG) treated with pyridostigmine bromide (PYR) with that of dogs treated with mycophenolate mofetil (MMF) and PYR (MMF + PYR).

Design—Retrospective case series.

Animals—27 dogs.

Procedures—Medical records from August 1999 through February 2008 were reviewed to identify dogs with serologically diagnosed acquired MG treated with PYR or MMF + PYR. Data collected for each dog included signalment, whether the dog had megaesophagus or pneumonia (or both), thyroid hormone concentration, remission, time to remission, and survival time. Rates for detection of clinical signs and survival time were compared. Survival time was estimated via the Kaplan-Meier method. Influence of drug treatment protocol on likelihood of remission, time to remission, and survival time was examined. Effects of MMF treatment, megaesophagus, pneumonia, and low serum thyroid hormone concentration on time to remission and survival time were also analyzed.

Results—12 dogs were treated with PYR, and 15 were treated with MMF + PYR. Mortality rates were 33% (PYR) and 40% (MMF + PYR). There was pharmacological remission in 5 and 6 dogs in the PYR and MMF + PYR groups, respectively. No significant differences were detected between treatment groups for remission rate, time to remission, or survival time. Megaesophagus, pneumonia, and low serum thyroid hormone concentration had no significant effect on time to remission or survival time for either treatment group.

Conclusions and Clinical Relevance—The results did not support routine use of MMF for the treatment of dogs with acquired MG. (J Am Vet Med Assoc 2010;236:664–668)

Acquired MG is an autoimmune neuromuscular disorder of dogs, the effective treatment for which can be challenging. Autoantibodies produced against the nicotinic AChR of skeletal muscles can impair neuromuscular transmission and cause clinical signs of weakness. Megaesophagus is a common finding in dogs with acquired MG and is attributed to the high proportion of skeletal muscle in the canine esophagus. Several clinical forms of acquired MG have been identified, including focal, generalized, acute fulminating, and paraneoplastic forms. Although many dogs with acquired MG may have remission of the disease without treatment, other dogs appear to require immuno-suppressive treatment as a result of persistence of the myasthenic condition. In addition, immunosuppressive treatment is often used in dogs whose clinical status is rapidly deteriorating despite anticholinesterase (eg, pyridostigmine) treatment. Of particular concern in dogs with acquired MG is the risk of fatal aspiration pneumonia associated with megaesophagus.

Despite the general consensus among veterinarians that dogs with acquired MG that are unresponsive to anticholinesterase treatment should receive immunosuppressive treatment, there is no consensus with regard to treatment recommendations. In addition, to our knowledge, no clinical trials (controlled or uncontrolled) have been conducted to evaluate the efficacy of the various immunosuppressive drugs typically used in the management of acquired MG in dogs. To date, clinical information has been limited to case reports and retro-

Abbreviations

AChR: Acetylcholine receptor
IMPDH: Inosine monophosphate dehydrogenase
MG: Myasthenia gravis
MMF: Mycophenolate mofetil
PYR: Pyridostigmine bromide
T₄: Thyroxine

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Mycophenolate mofetil is the prodrug form of mycophenolic acid. It is a selective inhibitor of the enzyme IMPDH, which is required in de novo cellular purine synthesis. In contrast to most cell lines, lymphocytes are unable to use the salvage pathway for purine synthesis; this causes lymphocytes to be dependent on IMPDH activity and the de novo pathway. In addition, there are 2 isoforms of IMPDH (ie, types I and II). The type II isoform is more prevalent than is the type I isoform in proliferating lymphocytes and is 3 to 5 times as sensitive to inhibition by mycophenolic acid as is type I. Mycophenolic acid inhibits T- and B-cell proliferation and decreases the amount of antibody production. Because acquired MG is a T-cell–dependent, B-cell–mediated process, MMF has been proposed for the treatment of acquired MG in dogs. The prevention of organ rejection in transplant patients has been the most common use of MMF in humans; however, there has been increased interest for the use of MMF in dogs with autoimmune disorders. Information regarding the use of MMF in dogs with acquired MG is restricted to a single case report in which the health of the dog appeared to improve as a result of treatment. The purpose of the study reported here was to compare the clinical outcome in dogs with serologically diagnosed acquired MG treated with PYR with that in dogs treated with MMF and PYR (MMF + PYR).

Materials and Methods

Case selection—Medical records were obtained from the following institutions: Cornell University, North Carolina State University, Texas A&M University, University of Georgia, Pet Emergency and Specialty Center of Marin, and the Veterinary Internal Medicine Practice of Northern Virginia. Qualifying records from August 1999 through February 2008 were reviewed to identify dogs with acquired MG. The diagnosis of acquired MG in all dogs was based on a negative cutoff concentration less than the reference range on time to MG remission and survival time were determined via backward stepwise Cox proportional hazards regression analysis. A value of P < 0.05 was considered significant for all analyses.

Results

Medical records of 27 dogs were reviewed. Twelve dogs were treated with PYR alone, and 15 were treated with MMF + PYR. Dogs in the PYR group ranged from 1 to 12 years old (mean, 5.5 years; median, 5 years), whereas dogs in the MMF + PYR group were 1 to 13 years old (mean, 6.8 years; median, 6 years). With the exception of 1 dog that was transiently (24 days) and unsuccessfully treated (worsening of clinical condition and maintained MG was continued in that dog. Immune remission was defined as the combination of clinical remission with the return of the AChR antibody titer concentration to < 0.6 nM/L without concurrent treatment directed against the acquired MG condition. Death, if attributed to the secondary effects of MG, was included for estimates of mortality rates.

Statistical analysis—Mortality and remission rates were compared between treatments by use of the Fisher exact test. The AChR antibody titer were compared between treatments by use of a Wilcoxon signed rank test. The Kaplan-Meier product limit method was used to estimate survival by treatment group. Log-rank analysis was used to compare treatment groups for resolution of megaesophagus, pharmacological or immune remission, time to remission (pharmacological or immune), and outcome (number of months from diagnosis until death); additionally, the same analysis was performed to compare remission and outcome in dogs with the generalized form of MG between the 2 treatment groups. Time from diagnosis of MG to initiation of treatment was also compared between treatment groups by use of a Mann-Whitney U test. In addition, effects of an AChR antibody titer, megaesophagus, pneumonia, and serum T4 concentration less than the reference range on time to MG remission and survival time were determined via backward stepwise Cox proportional hazards regression analysis. A value of P < 0.05 was considered significant for all analyses.

Mortality and remission rates
form in the MMF + PYR group, had radiographic evidence of a mass in the cranial aspect of the mediastinum that was suspected to be a thymoma. At the time of diagnosis, the serum AchR antibody concentrations for dogs in the PYR group ranged from 1.1 to 14.35 nM/L (mean, 4.17 nM/L; median, 2.51 nM/L); for dogs in the MMF + PYR group, these antibody concentrations ranged from 0.96 to 14.4 nM/L (mean, 3.70 nM/L; median, 2.98 nM/L). No significant ($P = 0.76$; Wilcoxon signed rank test) difference was detected in AchR antibody titers between the treatment groups. Megasosophagus was detected in 11 of 12 dogs treated with PYR and 13 of 15 dogs treated with MMF + PYR. Pneumonia was diagnosed in 8 dogs treated with PYR and 8 dogs treated with MMF + PYR. An endophthalmitis chloride response test was performed in 7 dogs treated with PYR (1 with the acute fulminating and 6 with the generalized forms) and 9 dogs treated with MMF + PYR (7 with the generalized and 2 with the acute fulminating forms); results of this test were considered negative in all dogs with the acute fulminating form of acquired MG. Serum T_{4} concentration was determined in 4 dogs treated with PYR and 11 dogs treated with MMF + PYR; T_{4} concentrations were less than the reference range (1 to 4 µg/dL) in 2 of the dogs treated with MMF + PYR (0.33 and 0.54 µg/dL, respectively). Except for 1 dog that received a continuous rate infusion, PYR was administered at 8- or 12-hour intervals to dogs in both treatment groups. Dosages in the PYR group ranged from 0.04 to 2.0 mg/kg (0.018 to 0.91 mg/lb), with a mean of 1.1 mg/kg (0.5 mg/lb) and a median of 0.97 mg/kg (0.44 mg/lb). Dosages of PYR in the group treated with MMF + PYR ranged from 0.3 to 2.4 mg/kg (0.14 to 1.09 mg/lb), with a mean of 1.1 mg/kg and a median of 0.8 mg/kg (0.36 mg/lb). Dosages of MMF ranged from 4 to 27 mg/kg (1.8 to 12.3 mg/lb), with a mean of 16.2 mg/kg (7.36 mg/lb) and a median of 20 mg/kg (9.09 mg/lb). Estimates for the time interval from diagnosis of the acquired MG condition to initiation of treatment were available for 11 dogs treated with PYR and 14 dogs treated with MMF + PYR. The number of days that dogs were treated with PYR ranged from 2 to 16 days (mean, 9.2 days; median, 8 days); the number of days that dogs were treated with MMF + PYR ranged from 1 to 75 days (mean, 12.6 days; median, 5.5 days). No significant ($P = 0.31$; Mann-Whitney U test) difference was detected between treatment groups for the likelihood of resolution of clinical signs of acquired MG (PYR, 5/12 [42%] dogs; MMF + PYR, 6/15 [40%] dogs), time to resolution of clinical signs ($P = 0.90$; log-rank test), or survival time ($P = 0.64$; log-rank test). Kaplan-Meier curves were constructed for clinical signs and survival time (Figures 1 and 2). For the generalized form of acquired MG in dogs, there was no difference ($P = 0.81$; log-rank test) between treatment groups for time to resolution of clinical signs ($P = 0.74$; log-rank test) or survival time ($P = 0.81$; log-rank test). Dogs that did not achieve pharmacological remission during treatment had greater AchR antibody concentrations at the time of diagnosis than did those that achieved pharmacological remission during treatment ($P = 0.03$; Kruskal-Wallis test); however, AchR antibody concentrations at the time of diagnosis were not significantly different ($P = 0.30$; Kruskal-Wallis test) between survivors and nonsurvivors. Mortality rate was not significantly ($P = 0.70$; Fisher exact test) different between dogs treated with PYR (mortality rate, 33.3% [4/12 dogs]) and dogs treated with MMF + PYR (mortality rate, 46.7% [7/15 dogs]). There was no significant difference ($P = 1.0$; Fisher exact test) detected between treatment groups for the likelihood of resolution of clinical signs of acquired MG (PYR, 5/12 [42%] dogs; MMF + PYR, 6/15 [40%] dogs), time to resolution of clinical signs ($P = 0.90$; log-rank test), or survival time ($P = 0.64$; log-rank test). Kaplan-Meier curves were constructed for clinical signs and survival time (Figures 1 and 2). For the generalized form of acquired MG in dogs, there was no difference ($P = 0.81$; log-rank test) between treatment groups for time to resolution of clinical signs ($P = 0.74$; log-rank test) or survival time ($P = 0.81$; log-rank test). Dogs that did not achieve pharmacological remission during treatment had greater AchR antibody concentrations at the time of diagnosis than did those that achieved pharmacological remission during treatment ($P = 0.03$; Kruskal-Wallis test); however, AchR antibody concentrations at the time of diagnosis were not significantly different ($P = 0.30$; Kruskal-Wallis test) between survivors and nonsurvivors.
A backward stepwise Cox proportional hazards analysis revealed that for the interaction of treatment with MMF; megaeosophagus and pneumonia had no significant effect on the number of months until resolution of clinical signs of disease (MMF, \( P = 0.92 \); megaeosophagus, \( P = 0.17 \); and pneumonia, \( P = 0.20 \)) or survival times (MMF, \( P = 0.52 \); megaeosophagus, \( P = 0.52 \); and pneumonia, \( P = 0.28 \)). Similarly, Cox proportional hazards analysis for the subset of dogs in which concentrations for \( T_4 \), free \( T_4 \), or both were available (PYR, \( n = 4 \); MMF + PYR, 11) revealed no effect of subnormal serum \( T_4 \) concentration on the number of months until resolution of clinical signs of disease (\( P > 0.9 \)) or survival times (\( P > 0.90 \)). Elevated AChR antibody titers at the time of diagnosis were associated with a decrease in the likelihood of pharmacological remission (hazard ratio, 0.53 [95% confidence interval, 0.29 to 0.97]; coefficient, \(-0.64 \ [P = 0.04]\) ; goodness-of-fit model, \(-2\); log likelihood, 47.2; \( \chi^2 = 8.08 \ [P = 0.005]\) ) and a nonsignificant increase in the risk of death (hazard ratio, 1.18 [95% confidence interval, 1.01 to 1.37]; coefficient, \(0.16 \ [P = 0.04]\) ; goodness-of-fit model, \(-2\); log likelihood, 60.5; \( \chi^2 = 3.58 \ [P = 0.058]\) ). These results suggest that for every increase of 1nM/L in AChR antibody concentration at the time of diagnosis, dogs were 1.18 times as likely to die and 0.53 times as likely to achieve pharmacological remission at any point during treatment.

Adverse effects (eg, drooling, vomiting, or soft feces) as a result of treatment were reported for 6 of 12 dogs in the PYR treatment group; similarly, adverse effects were reported for 10 dogs in the MMF + PYR treatment group. The reduction or resolution of adverse effects on the gastrointestinal tract coincided with a reduction in the MMF dose.

**Discussion**

The treatment of acquired MG in dogs can be challenging. Currently, there is a paucity of controlled and uncontrolled studies conducted to clearly determine the immunosuppressive treatment that is of most benefit. A similar situation exists in the treatment of MG in humans. The benefit of MMF in the treatment of acquired MG in humans is controversial. In contrast to the medical management of acquired MG in dogs, prednisone is considered the immunosuppressive treatment of choice for acquired MG in human patients. Typically, evaluation of the efficacy of other immunosuppressive drugs for acquired MG in humans is accomplished by comparing results for prednisone-treated patients with results for patients treated with prednisone and an investigational drug. The investigational drug is typically prescribed as an adjunct to prednisone treatment, which may also reduce the dose of prednisone. Case reports, retrospective case series, and open-label uncontrolled studies suggest that MMF use is beneficial in the treatment of acquired MG in humans. However, 2 randomized, double-blinded, placebo-controlled prospective studies did not find a significant benefit from the administration of MMF over prednisone for the treatment of acquired MG in humans. In contrast to humans with acquired MG, there appears to be a substantial percentage of dogs with acquired MG that will undergo disease remission while only being treated with PYR.

Therefore, dogs being treated because of acquired MG are treated with either PYR alone or PYR with 1 or more immunosuppressive drugs (other than glucocorticoids). Because the dogs in the control group (ie, PYR group) of the present study did not receive an immunosuppressive drug, our study differs from comparative studies of acquired MG in humans.

Two dogs from each treatment group, which were being treated for acute fulminating MG, died shortly after hospital admission. There was no difference between treatment groups for remission or mortality rates. Because most dogs in both treatment groups had the generalized form of MG, dogs with the generalized form were separately compared by treatment group for remission and mortality rates; no difference was detected between treatment groups for dogs that had the generalized form of MG. Analysis of the results of our retrospective case series does not support the routine use of MMF for the treatment of dogs with acquired MG.

Randomized studies have detailed a significant lack of efficacy of MMF; however, MMF has been commonly used and generally has been considered a safe and effective treatment option for acquired MG in humans. Adverse effects localized to the gastrointestinal system have been reported. In humans treated with MMF; these effects are detected in a minority of treated patients, are not generally considered major effects, and typically are reversible with a reduction in dose of MMF. Although similar adverse effects appeared to be dose related, these effects were detected in 18 of 27 (67%) dogs treated with MMF in the present study. Even though this was a self-limiting phenomenon, the likelihood that MMF treatment would cause a disturbance in the gastrointestinal tract of dogs needs to be considered as well as the apparent overall lack of efficacy of MMF for controlling the myasthenic condition.

![Figure 2—Kaplan-Meier curve comparing survival over time in 27 dogs with acquired MG receiving treatment with PYR alone or MMF + PYR. See Figure 1 for remainder of key.](image-url)
Limitations are associated with our study, most of which are disadvantages of a retrospective case series. In addition to the relatively small number of cases, dogs were not randomly assigned to treatment groups. Because veterinarians typically include MMF as a treatment in dogs that exhibit an initial poor response to PYR alone, the MMF + PYR group may have been biased by dogs with acquired MG that were more refractory to treatment. Because 2 dogs treated with PYR achieved sustained immune remission, whereas the acquired MG condition relapsed in 3 dogs treated with MMF + PYR during the withdrawal phase of MMF treatment, the concept that the MMF + PYR group may have consisted of dogs with acquired MG that was more difficult to treat could be supported. Even if we assume this concept is correct, despite not having a comparative group of dogs, a pharmacological remission rate of only 6 of 13 (40%) and a mortality rate of 46.7% do not provide support for including MMF during treatment of dogs with refractory MG. Furthermore, we cannot predict the mortality rate in a group of dogs with acquired MG in which MMF was not administered. The treatment groups compared in the present study were similar with respect to signalment, serum AChR antibody concentrations, and distribution of clinical forms of acquired MG; however, there was a substantial range in the doses administered for both PYR and MMF in each group, which could have affected the outcome of the treatments. Ideally, a prospective, double-blinded, placebo-controlled study should be conducted to evaluate MMF treatment in dogs with acquired MG. Because of the risk of death in dogs with poorly controlled acquired MG and the lack of an accepted standard immunosuppressive drug for treating this disease in dogs, such an investigation poses an ethical dilemma.

An interesting finding of the present study, unrelated to drug regimen, was the inverse relationship between AChR antibody titer at the time of diagnosis and likelihood of attaining disease remission for all dogs. This finding may have some clinical use when planning a treatment strategy, frequency of reevaluation of AChR antibody concentrations, and rate of drug withdrawal. In this retrospective case series, no significant benefit was detected in the treatment of dogs with acquired MG by administration of MMF + PYR versus PYR alone. Clinicians need to interpret the results of this investigation cautiously for several reasons, which involve the small number of dogs included in this investigation, the lack of randomization to treatment groups, and the variability of disease severity among the dogs and dose of drugs administered.

References