HUMAN INTRAVENOUS IMMUNOGLOBULIN (IVIG) THERAPY IN VETERINARY MEDICINE – A REVIEW

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INTRODUCTION

Intravenous immunoglobulin (IVIG) is a therapeutic biological product containing highly purified polyspecific IgG obtained from pooled plasma on a large scale (3000 to 100,000 healthy blood donors) (1-3). Donors are screened for antibodies for human immunodeficiency virus (HIV), hepatitis B and C and human T cell lymphotrophic retroviruses (HTLV). Other measures such as treatment with solvents and detergents, trypsin, pasteurization, nano-filtration, and low pH are used to minimize the possibility of transmitting infectious disease agents.

The final product should contain more then 90% IgG (all subclasses) and only traces of IgM and IgA. Osmolality may range from physiological values (280-296 mOsm/l) to greater then 1,000 mOsm/l and most of the products have a pH value of 6-7.

IVIG treatment was introduced in the 1950's as a replacement therapy for congenital humoral immunodeficiency syndromes, and since then IVIG has proved to have immune modulating properties and successful in treating inflammatory, immune-mediated and autoimmune diseases. Common clinical indications include different immunodeficiencies such as hypogammaglobulinemia, pediatric HIV infection and B cell chronic lymphocytic leukemia. IVIG treatment has also been found beneficial in different immune-mediated diseases such as idiopathic thrombocytopenia purpura (ITP), autoimmune hemolytic anemia (AIH), autoimmune neutropenia, pure red cell aplasia, Kawasaki syndrome (severe childhood vasculitis), prevention of graft-versus-host disease in bone marrow recipients, chronic inflammatory demyelination polyneuropathy (CIDP), Guillain-Barre syndrome (acute, autoimmune polyradiculoneuropathy) and in other conditions (1-3).

At a recommended dose of 1g/kg and a production cost of US$50 per gram (500 US$/10kg dog), the high cost may hinder its routine use for therapy in veterinary medicine. Moreover, until now, its cost and the uncertainty of its usefulness have hindered controlled randomized clinical trails in veterinary medicine.

No canine or feline origin intravenous immunoglobulin product is currently available on the market due to its high cost of production for a potentially small market. Moreover, no serious adverse effects have been detected following the use of human immunoglobulin in dogs or cats. For pet owners who are willing to pay the high price, this novel therapy may make the difference between life and death.

Immune modulation: Mechanism of action

The exact mechanism by which IVIG modulates the immune system is not fully understood, although there is evidence to support several hypotheses (2, 12-14).

IVIG modulates both humoral and cell immunity in many locations, different cell types, different receptors and different effector molecules (4, 1-3). IVIG preparations can activate/block Fc receptors. IgG Fc receptors (FcγRs) are transmembrane glycoproteins of macrophages, neutrophils, eosinophils, platelets, mast cells, natural killer cells and B lymphocytes (1). Binding of IgG molecule at its Fc region may modify many functions, depending on the type of the effector cell. Such enhanced functions are phagocytosis, degranulation, antibody-dependent cell-mediated toxicity, cytokine release, and regulation of antibody production.

It is accepted that the main mechanism of action of IVIG in the treatment of ITP and other immune mediated cytopenias is mediated through the blockade of macrophages FcγRs, leading to a slower removal rate of antibody coated platelets (1, 3). In a canine *in vitro* model, it has been shown that monocytes bind IVIG through the Fc receptor and that this mechanism of action leads to a slower removal rate of antibody coated erythrocytes; these authors also found that IVIG binds to B, CD4 and CD8 lymphocytes (4). Other immune modulation properties include different antigen binding, attenuation of complement mediated damage, neutralization of pathologic auto-antibodies and induction of anti-inflammatory cytokines. IVIG can bind to different antigens including natural antibodies, which are not the result of an immune response and are present even in the embryo and gnotobiotic animals. These natural antibodies are a part of the innate immune system but they can also behave as reactive auto-antibodies, promoting autoimmunity (1).

IVIG contains antibodies to super-antigens that may accelerate viral or bacterial induced autoimmune manifestations; it also contains antibodies against CD4 and MHC class 1 which block the function of CD4 positive lymphocytes and CD8 lymphocytes, respectively. Moreover natural antibodies against different pro-inflammatory cytokines are down regulated by IVIG.

Another mechanism involves the attenuation of complement-mediated damage by activated C3b and C4b, and thus IVIG prevents the C5b-9 membrane attack complex (1). This mode of action is very importance in the treatment of dermatomyositis, Guillain-Barre syndrome and myasthenia gravis. IVIG is able to bind pathogenic auto-antibodies and to induce the production of anti-inflammatory cytokines by T helper I & II such as interleukin-1 receptor antagonist (1-3).

Immune mediated thrombocytopenia (IMT)

Canine immune mediated thrombocytopenia refers to any

thrombocytopenia that results from immunological destruction of platelets, usually by means of phagocytosis of antibody coated platelets by the mononuclear phagocytic system (5). IMT can be primary (i.e. autoimmune) or secondary to a primary condition such as neoplasia, infectious agents, systemic autoimmune disease and drug or toxin exposure.

Idiopathic thrombocytopenia purpura (ITP) was the first immune mediated disease to be successfully treated by IVIG in human medicine. Imbach et al. (1981) showed that high doses of IVIG could reduce the destruction of platelets in a child suffering from ITP. Since then IVIG therapy has been reconfirmed in many clinical trails, and ITP is one of the best established clinical indications..

Since IVIG is very expensive, and the fact the canine IMT usually responds well to common immunosuppressive and immuno-modulating drugs such as glucocorticoids, vincristine, azathioprine, cyclosporine, danazole, cyclophosphamide and leflunomide, only a few case studies were published with canine IMT but no controlled randomized clinical trials. Another major fault connected with its high cost is that only severe, refractory cases have been treated with IVIG, which compromises the ability to evaluate its clinical relevance.

Only two case studies have been published in the veterinary literature regarding IVIG therapy in canine IMT. In an early study one dog with a presumed diagnosis of primary IMT was reported (6). The dog was treated with 2 mg/kg of prednisone every 12 hours for 11 days without improvement. Prior to IVIG therapy the platelet count was 5000 cells/µL. IVIG was administrated at a dose of 1 g/kg over a period of 12 hours. During this period and thereafter, prednisone therapy was continued. Six hours after IVIG was infused the platelet count rose to 70,000 cells/µL; 24 hour later platelet count was 145,000 cell/µL, and 4 days later it was 240,000 cells/µL. The dog relapsed on day 23 post treatment with a platelet count of 57,000 cells/µL. The authors of this study did not document any signalment, medical history, physical examination or clinical pathological data such as CBC, blood smear examination, serum chemistry and bone marrow analysis of the dog. In the same study it was noted that 4 dogs that were diagnosed with immune mediated hemolytic anemia (IMHA) and were treated with IVIG had concurrent thrombocytopenia. In all 4 dogs platelets count returned to the reference range within 1 to 16 days; however 3 dogs relapsed with thrombocytopenia. In a more recent study 5 dogs that were refractory to conventional therapy were treated with IVIG (7). All the dogs underwent a thorough clinical and laboratory investigation including physical examination, CBC and blood smear examination, serum biochemistry profile, coagulation panel (including PT/aPTT/FDPs and fibrinogen), serological tests for tick borne disease and bone marrow core biopsy and cytology.

All dogs were extremely thrombocytopenic (< 3,000 cells/µL) before IVIG infusion. IVIG was infused in a dose of 0.28-0.76 g/kg over a six hours period. No adverse reactions were noted during and after treatment in any of the dogs. Three of the dogs had dramatically improved thrombocyte counts (12,000 – 68,000 cells/µL) immediately after the treatment. One dog had a platelets count of 6,000 cell/µL immediately after the treatment, but 24 hours later platelets count rose to 58,000 cells/µL. Only one dog did not respond immediately and vincristine therapy was initiated. None of the dogs relapsed over the follow up period (6 months).

Immune mediated hemolytic anemia

Canine immune mediated hemolytic anemia (IMHA) is relatively a common life threatening, hematological disorder. Its pathogenesis comprises auto-antibody formation against mature erythrocytes or erythrocytes precursors, complement activation and hemolysis (8).

Hemolysis may be intravascular as a result of the formation of membrane attack complexes, and as a consequence, osmotic destruction of erythrocytes, but more frequently it occurs in an extravascular location (mainly in the spleen or liver) by means of phagocytosis of antibody coated erythrocytes by the mononuclear phagocytic system. The efficacy of IVIG treatment in canine primary IMHA was evaluated (9-11, 6, 12), most of the studies were published more than ten years ago, and no new information is available.

In the only prospective clinical trial, 10 dogs that were refractive to conventional immunosuppressive drugs were treated with IVIG (6). The authors concluded that IVIG therapy had a short term effect, and hematocrit, hemoglobin and reticulocytes counts improved significantly over a short period of time but did not last for the long term, and only 3 dogs were alive one year after the first clinical presentation. Nevertheless one should bear in mind that only the cases that were refractive to common drug therapy were treated, i.e., cases that had a poor prognosis at the outset.

In another retrospective study (6), 13 dogs with primary IMHA that were responding to immunosuppressive therapy were treated with IVIG. Out of the 13 dogs 10 were alive for discharge after sufficient improvement in their hematocrit. Out of these 10 dogs 8 were still alive after a median of 460 days (range 120-1,075 days). The authors suggested that IVIG therapy should be considered in more severe cases of canine IMHA despite its high cost which should be interpreted in light of the shorter time of hospitalization and the potential decrease in the use of blood product transfusions.

More prospective, large scale clinical trials with longer follow up periods are needed before recommending the use of IVIG in the treatment of refractive canine IMHA patients.

Myelofibrosis

Myelofibrosis is characterized by deposits of fibrous tissue in the extracellular matrix of the bone marrow by reactive marrow fibroblasts, hindering normal hematopoiesis (13). Transforming growth factor β (TGF- β), platelet derived growth factor (PDGF) and epidermal growth factor (EGF) are fibrogenic cytokines that are implicated as important modulators of proliferation and synthesis in marrow fibroblasts and endothelial cells. Myelofibrosis is a reactive process associated with various diseases and can be resolved by the correction of the primary condition.

In the cat the most common underling cause for myelofibrosis

is myelodysplasia and acute myeloid leukemia while in the dog it is mostly associated with immune mediated, non-regenerative anemia. Affected dogs are anemic and reticulocytopenic despite significant, but ineffective, erythroid hyperplasia. Myelofibrosis also occurs in dogs with hemolytic anemia due to pyruvate kinase deficiency. Other underlying causes include infectious diseases, radiation, drugs and cancer, with or without marrow involvement.

In a case study of dogs with myelofibrosis and a suspected immune mediated underlying disease were treated with IVIG. Doses, pre- and post- IVIG hematocrit, reticulocyte counts, duration of response and outcome are shown in table 1 (taken from the original paper) (6). Clearly, much more information is needed before recommending IVIG therapy in myelofibrosis.

Table 1. Dose, response and outcome of IVIG treatment of five dogs with nonregenerative anemia and myelofibrosis

Case	Dose of IVIG (g/kg)	Pre-IVIG Hematocrit (%)	Post-IVIG hematocrit (peak)	Pre-IVIG reticulocyte count (/µL)	Post-IVIG reticulocyte count (/µL)	Duration of response (days)	Outcome
1	1.5	9.1	28 (day 10)	42,000	378,000	262	Euthanized (PCV= 26%)
2	1.5	6.6	17 (day 27)	22,000	306,000	37	Retreated
3	1.5	7.7	18 (day 24)	21,000	199,000	49	Retreated
4	1	16	31 (day 11)	22,000	550,000	37	Euthanized (PCV= 27%)
5	1	10	24 (day 47)	35,000	474,000	159	Relapsed (PCV=12%)

Pemphigus foliaceus

Pemphigus foliaceus (PF) is the most common immune mediated skin disease of the dog. Its pathogenesis includes the formation of auto-antibodies against desmogelin 1, a keratinocyte desmosome component (14-16). The desmosomes are the site of adhesions between keratinocytes. Binding of antibodies result in breakdown of adhesions between keratinocyes, acantholysis, subcorneal blister formation and pustules.Conventional therapy includes immunosuppressive doses of corticosteroids and other cytotoxic and immunosuppressive drugs such as azathioprine, cyclophosphamide, cyclosporine, chlorambucil and gold therapy.

In a case report by Rahilly et al (2006) a single dog with severe PF was treated with IVIG (16). IVIG therapy was initiated before other immunosuppressive drugs were given. After the initial dose of 0.5 g/kg there was a marked improvement of clinical signs, skin lesions became drier, erythema began to resolve and no new skin lesion were noted at 12 days post IVIG therapy, moreover the dog became stronger and showed an interest in food. The initial dose was then repeated three more times in the next 4 days (total dose of 2 g/kg) following by immunosuppressive therapy (prednisone 2.2 mg/kg PO q24h and azathioprine 2 mg/kg PO q24h). The dog was discharged from hospitalization but with remission of clinical signs 2 days after the initiation of the immunosuppressive therapy. Four weeks later another dose of IVIG (0.5 g/kg) was given. The dog remained in remission for 9 weeks post hospitalization when the disease relapsed with new skin lesions, fever and weakness. The dog received an additional 2 doses of IVIG (0.5 g/kg each) in the next 2 days and azathioprine therapy was discontinued. Another single dose (0.5 g/kg) of IVIG was given on weeks 12, 22, 26 and 31. The dog remained in remission for 1 year after the initial diagnosis and 4.5 months after the last IVIG therapy.

In a resent review study of 91 dogs with PF by Muller et al. (2006), the mean average time to remission was 9.3 months (range 1-36 months (15). It was noted that dogs treated with a combination therapy of prednisone and azathioprine had longer remission periods compared to the dogs treated with prednisone alone (11 months vs. 7 months), though there was no difference between the groups regarding the initial response to therapy. In an earlier review study by Gomez et al (2004) the authors found a case fatality rate of 60.5% (14). In the survival group the duration of treatment ranged from 6-63 months where 10 out of the 17 survivors continued to receive treatment. Of the non-survivors 92% had a survival time of less than 12 months.

Treatment of adverse cutaneous drug reactions

Severe, adverse cutaneous drug reactions are rare in the dog and the cat and may include erythema multiforma (EM), Steven-Johnson syndrome (SJS), toxic epidermal necrolysis and Stevens-Johnson epidermal necrolysis overlap syndrome.

The basis of these syndromes is the detachment of the epidermis from the dermis, probably due to immune mediated processes, and the formation of erythematous macular dermatitis with focal to widespread mucocutaneous vesicles and ulceration. Patients more severely affected are usually pyrexic, depressed and prone to secondary infections. Prognosis is grave in severe cases but can be good in the more localized and non-systemic affected animals. Treatment usually comprises of withholding the offending drug and giving aggressive supportive care; standard immunosuppressive drugs such as glucocorticoids and azathioprine are not of value in these cases.

There have been three case reports in the veterinary literature concerning the treatment of 3 dogs and one cat with severe adverse cutaneous drug reaction with IVIG. The first case reported by Byrne et al (2002) was of a 5 months old cat that developed extensive crusts and a single ulcerated fissure with purulent exudates (17). The cat was febrile, lethargic and thin. A tentative diagnosis of EM was established upon history, clinical signs, clinical pathology and histopathology. Because of the extent and severity of clinical signs, the lack of response to supportive care and the progressive nature of EM, treatment with IVIG was initiated. IVIG was infused at 1gr/kg dose over a 4-hour period b.i.d. for two days. Eight days later the cat was reevaluated and was bright, alert, afebrile and most of the skin lesion had progressively improved with >90% resolution of the crusts. Fifty-seven days later the cat returned for routine ovariohysterectomy. The cat showed no remarkable findings on physical examination and had gained 1.4 kg (100% rise in body weight), CBC, serum biochemistry and urinalysis results were all within the reference range.

The second case reported was of a 2 year old dog that had developed severe mucocutaneous ulceration, consistent with SJS, after trimethoprim-potentiated sulphadiazine therapy. Systemic signs included severe hepatopathy, dyspnea, pyrexia cachexia, and keratoconjunctivitis sicca (KCS) was also noted. Aggressive supportive care did not improve the dog's condition, moreover, glucocorticoid therapy probably lead to *Pseudomonas aeruginosa* infection of the nasopharynx. Due to the deterioration and the unresponsive state of the dog, a single infusion of 0.51 g/kg IVIG was initiated. Treatment after the IVIG infusion consisted of antimicrobial therapy and topical liquid paraffin ointment. All clinical sign resolved and the dog did not relapse.

Two cases were reported by Trotman et al (2006) were of dogs suffering from severe adverse cutaneous drug reactions (18). Both were refractory to aggressive supportive therapy, and were treated with 2 doses of 1gr/kg IVIG in two consecutive days. In both cases all clinical signs resolved and no relapse was noted in the follow-up period of more than 3 years.

Side effects

Adverse reaction during, and after, IVIG infusion are uncommon events in human patients and rare in veterinary patients. Common mild side effects reported in human medicine are fever, headache, chills, back pain, nausea, vomiting, tachycardia, shortness of breath, and chest tightness. More severe reactions may include an anaphylactic reaction to IgA in IgA-deficient patients, hemolytic anemia due to blood group antibodies, viral contamination, aseptic meningitis, acute renal failure due to osmotic nephrosis and thrombotic episodes in elderly patients treated for ITP.

No major side effects have been noted in veterinary patients. One normal dog vomited during IVIG infusion. Mild thrombocytopenia was recorded in three normal dogs after IVIG infusion, another 3 dogs treated for IMHA that were not thrombocytopenic prior to therapy became thrombocytopenic 1 to 6 days later with a nadir of 32,000 to 189,000 cells/µL, though it should be noted that thrombocytopenia is a well documented complication of canine IMHA. No adverse reactions have been reported in more recent case reports.

Summary

IVIG is a novel promising therapy for a wide verity of immune mediated diseases with the potential of long-term remission. In human medicine only a few indications are approved by the FDA after controlled randomized clinical trails have shown efficacy.

These indications are:

- Allogenic bone marrow transplant
- Chronic B lymphocytic leukemia
- Idiopathic thrombocytopenia purpura
- Pediatric HIV
- Primary immunodeficiencies
- Kawasaki diseases.
- Host vs. graft disease

But many more off-label indications are used today.

In veterinary medicine only a few immune mediated diseases were evaluated for their response to IVIG therapy but there are no approved indications. Nevertheless, its use may be indicated in a wide variety of severe unresponsive immune mediated diseases.

REFERENCES

1. Boros, R, Gondolesi, G. and Bromberg, J.S.: High dose intravenous immunoglobulin treatment: mechanisms of action. Liver Transpl. 11:1469-1480, 2005.

2. Lemieux, R., Bazin, R. and Neron, S.: Therapeutic intravenous immunoglobulins. Mol Immunol. 42:839-848, 2005.

3. Nimmerjahn, F. and Ravetch, J.V.: The antiinflammatory activity of IgG: the intravenous IgG paradox. J Exp Med. 204:11-15, 2007.

4. Reagan, W.J., Scott-Moncrieff, C., Christian, J., Snyder, R, Kelly, K. and Glickman, L.: Effects of human intravenous immunoglobulin on canine monocytes and lymphocytes. Am J Vet Res. 59:1568-1574, 1998.

5. Scott, M.A., *Immune-Mediated Thrombocytopenia*, in Schalm's Veterinary Hematology, B.F. Feldman, J.G. Zinkl, and N.C. Jain, Editors. 2000, Lippincot Williams and Wilkins: Philadelphia. p. 478=487.

6. Scott-Moncrieff, J.C., Reagan, W.J., Snyder, P.W. and Glickman, L.T.: Intravenous administration of human immune globulin in dogs with immune-mediated hemolytic anemia. J Am Vet Med Assoc. 210:1623-1627, 1997.

7. Bianco, D., Armstrong, P.J. and Washabau, R.J.: Treatment of severe immune-mediated thrombocytopenia with human IV immunoglobulin in 5 dogs. J Vet Intern Med. 21:694-699, 2007.

8. Day, M.J., *Immune-Mediated Hemolytic Anemia*, in *Schalm's Veterinary Hematology*, B.F. Feldman, J.G. Zinkl, and N.C. Jain, Editors. 2000, Lippincott Williams and Wilkins: Philadelphia. p. 799-807.

9. Scott-Moncrieff, J.C., Reagan, W.J., Glickman, L.T., DeNicola, D.B. and Harrington, D.: Treatment of nonregenerative anemia with human gamma-globulin in dogs. J Am Vet Med Assoc. 206:1895-1900, 1995.

10. Kellerman, D.L. and Bruyette, D.S.: Intravenous human immunoglobulin for the treatment of immune-mediated hemolytic anemia in 13 dogs. J Vet Intern Med. 11:327-332, 1997.

11. Scott-Moncrieff, J.C. and Reagan, W.J.: Human intravenous immunoglobulin therapy. Semin Vet Med Surg (Small Anim). 12:178-185, 1997.

12. Grundy, S.A. and Barton, C.: Influence of drug treatment on survival of dogs with immune-mediated hemolytic anemia: 88 cases (1989-1999). J Am Vet Med Assoc. 218:543-546, 2001.

13. Blue, J.T., *Myelodysplastic Syndromes and Myelofibrosis*, in *Schalm's Veterinary Hematology*, B.F. Feldman, J.G. Zinkl, and N.C. Jain, Editors. 2000, Lippincott Williams and Wilkins: Philadelphia. p. 682-689.

14. Gomez, S.M., Morris, D.O., Rosenbaum, M.R. and Goldschmidt, M.H.: Outcome and complications associated with treatment of pemphigus foliaceus in dogs: 43 cases (1994-2000). J Am Vet Med Assoc. 224:1312-1316, 2004.

15. Mueller, R.S., Krebs, I., Power, H.T. and Fieseler, K.V.: Pemphigus foliaceus in 91 dogs. J Am Anim Hosp Assoc. 42:189-196, 2006.

16. Rahilly, L.J., Keating, J.H. and O'Toole, T.E.: The use of intravenous human immunoglobulin in treatment of severe pemphigus foliaceus in a dog. J Vet Intern Med. 20:1483-1486, 2006.

17. Byrne, K.P. and Giger, U.: Use of human immunoglobulin for treatment of severe erythema multiforme in a cat. J Am Vet Med Assoc. 220:197-201, 183-194, 2002.

18. Trotman, T.K., Phillips, H., Fordyce, H., King, L.G., Morris, D.O. and Giger, U.: Treatment of severe adverse cutaneous drug reactions with human intravenous immunoglobulin in two dogs. J Am Anim Hosp Assoc. 42:312-320, 2006.