Adverse reactions suggestive of type III hypersensitivity in six healthy dogs given human albumin

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Case Description—6 healthy dogs given human albumin solution as part of a study were examined following development of an immediate hypersensitivity reaction (1 dog) and signs suggestive of a type III hypersensitivity reaction (all 6 dogs).

Clinical Findings—All 6 dogs were healthy prior to administration of human albumin solution. One dog developed signs of an immediate hypersensitivity reaction, characterized by vomiting and facial edema, during administration of human albumin solution. All 6 dogs developed signs of a delayed adverse reaction 5 to 13 days after administration of human albumin solution. Initial clinical signs included lethargy, lameness, edema, cutaneous lesions indicative of vasculitis, vomiting, and inappetance.

Treatment and Outcome—in the dog with signs of immediate hypersensitivity, signs resolved after administration of human albumin solution was discontinued and diphenhydramine was administered. Supportive treatment was provided after dogs developed signs of a delayed adverse reaction. Four dogs recovered, but 2 dogs died despite treatment. All 6 dogs were found to have antihuman albumin antibodies. There was no evidence of contamination of the human albumin solution.

Clinical Relevance—Findings suggest that administration of human albumin solution in healthy dogs with normal serum albumin concentrations may result in signs of a type III hypersensitivity reaction. (J Am Vet Med Assoc 2007;230:873–879)

Six healthy dogs were examined because of adverse reactions that developed following administration of human albumin solution. These dogs represented 6 of the 7 dogs enrolled in a prospective study designed to determine the effects of administration of 25% human albumin solution, 6% hetastarch, 10% pentastarch, normal saline (0.9% NaCl) solution, and a balanced electrolyte solution on coagulation parameters, PCV, serum total solids concentration, and colloid oncotic pressure in healthy dogs. The 7 dogs enrolled in the study were owned by students, staff members, or faculty members of the Washington State University Veterinary Teaching Hospital, who had provided informed consent for inclusion of their dogs in the study. The study protocol had been approved by the Washington State University Institutional Animal Care and Use Committee.

During the first phase of the study, the 6 dogs that were the subject of the present report (4 spayed females and 2 castrated males; median age, 2 years) received a single dose of 25% human albumin solution4 (2 mL/kg [0.9 mL/lb]). The human albumin solution was administered IV over 1 hour and was the first solution administered to these dogs during the study. The seventh dog did not receive human albumin solution.

During administration of the human albumin solution, one of the dogs (dog 1) developed signs of an immediate hypersensitivity reaction, characterized by vomiting and facial edema. Administration of human albumin solution was immediately discontinued, with the result that this dog received a total dose of only 0.5 mL/kg (0.23 mL/lb) over a 15-minute period. Signs resolved after administration of human albumin solution was discontinued, and diphenhydramine (2 mg/kg, IV) was given. The remaining 5 dogs did not develop any immediate adverse reactions.

All 6 dogs developed clinical signs of a delayed adverse reaction at various times after administration of human albumin solution. The onset of clinical signs ranged from 5 to 13 days after administration of human albumin solution. Initial clinical signs included lethargy (6 dogs), shifting limb lameness (5), forelimb edema (5), facial edema (4), hind limb edema (2), vulvar edema (1), cutaneous lesions indicative of vasculitis (5), vomiting (5), inappetance (4), ecchymoses (4), signs of depression (3), generalized lymphadenopathy (3), conjunctival erythema (3), diarrhea with hematochezia and melena (2), hematemesis (1), generalized pruritus (1), and atrophy of the temporalis and masseter muscles (1).

In 4 of the 6 dogs (dogs 1 to 4), clinical signs were of insufficient severity to warrant hospitalization; all 4 of these dogs survived. Treatment was nonspecific and included administration of dexamethasone (0.05

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During the first 4 days of hospitalization, dog 5 developed neutrophilia with a regenerative left shift, monocytosis, progressive anemia and thrombocytopenia, azotemia, prolonged coagulation parameters (1-stage prothrombin time and activated partial thromboplastin time), hypermagensemia, hypernatremia, hyperkalemia, and hyperchloremia; serum alkaline phosphatase activity progressively increased during this time. On the fourth day of hospitalization (16 days after administration of human albumin solution), the dog developed hypertension (systolic blood pressure, 190 to 255 mm Hg), which was treated with enalapril (0.4 mg/kg, PO, q 12 h). Treatment with heparin (100 U/kg [45.5 U/lb], SC, q 6 h) was initiated because of development of disseminated intravascular coagulation. Disseminated intravascular coagulation was diagnosed on the basis of high D-dimer concentration (1,000 to 2,000 ng/mL; reference range, <250 ng/mL) in combination with prolonged 1-stage prothrombin and activated partial thromboplastin times and thrombocytopenia. The dog was given 1 unit (7.5 mL/kg [3.4 mL/lb], IV) of cross-matched packed RBCs.

Punch biopsy specimens of cutaneous lesions were obtained from dog 5. Histologic examination revealed severe neutrophilic dermal vascular inflammation, and a diagnosis of leukocytoclastic vasculitis was made. On the basis of a presumptive diagnosis of type III hypersensitivity reaction with systemic vasculitis, treatment with cyclosporine (2.5 mg/kg [1.1 mg/lb], PO, q 12 h), azathioprine (2.5 mg/kg, PO, q 24 h), and prednisone (1 mg/kg, PO, q 12 h) was begun. These 3 drugs were used concurrently to suppress immune system function through several different mechanisms. Azathioprine is a purine antimetabolite that has its greatest effect on cellular immunity and delayed hypersensitivity reactions, with some effects on humoral immunity.1 Cyclosporine suppresses the cell-mediated immune response, particularly T helper cells, with minor effects on the humoral response.1 Prednisone, at the dosage administered, has its greatest effects on nonspecific immune responses, including neutrophil, macrophage, and monocyte migration; cytokine production; antigen processing; chemotaxis; phagocytosis; intracellular killing; and the complement cascade.1

Signs of oliguric acute renal failure with inadequate urine production (<1 mL/kg/h) developed. The rate of IV fluid administration was increased to 7.5 mL/kg/h, and dopamine was administered as a continuous-rate infusion (3 µg/kg/min) to improve renal perfusion. Treatment with cyclosporine was continued despite the evidence of renal failure because it was thought that resolution of immune complex formation was necessary before renal function would improve.

A CBC and serum biochemistry panel performed on the fifth day of hospitalization (17 days after administration of human albumin solution) revealed persistent neutrophilia with a regenerative left shift, monocytosis, progressive anemia and thrombocytopenia, azotemia, prolonged coagulation parameters (1-stage prothrombin time and activated partial thromboplastin time), hypermagensemia, hypernatremia, hyperkalemia, and hyperchloremia; serum alkaline phosphatase activity progressively increased during this time in serum alkaline phosphatase activity, hyperkalemia, hyperphosphatemia, hypermagnesemia, hyperchlo-
mia, and hyponatremia. Two peritoneal catheters were surgically placed on the fifth day of hospitalization, and peritoneal dialysis was performed multiple times during the next 2 days. Although urine production improved, evidence of blood loss progressed, as there was evidence of hemoabdomen and continued gastrointestinal tract blood loss. Two units (20 mL/kg, IV) of cross-matched fresh-frozen plasma and 500 mL (20 mL/kg, IV) of cross-matched fresh whole blood were transfused. Administration of enalapril was continued because of ongoing hypertension and development of proteinuria. Low-dose aspirin therapy was considered, but was ultimately avoided because of administration of prednisone at an immunosuppressive dosage and the ongoing evidence of gastrointestinal tract bleeding.

Laboratory results on the following day were similar to those obtained on the fifth day of hospitalization. Measurement of total bilirubin concentration revealed severe hyperbilirubinemia.

On the seventh day of hospitalization, harsh lung sounds were ausculted, and ventricular premature contractions and ventricular tachycardia were detected during continuous electrocardiographic monitoring, raising concerns about myocardial and pulmonary hemorrhage. Lung sounds were closely monitored, and a continuous-rate infusion of lidocaine (40 μg/kg/min [18 μg/lb/min], IV) was started to control the ventricular tachycardia. Phynadione (2.5 mg/kg, SC, q 24 h), 1 unit (10 mL/kg [4.5 mL/lb], IV) of cross-matched fresh-frozen plasma, 1 unit of cross-matched packed RBCs (7.5 mL/kg, IV), and 180 mL (7.5 mL/kg, IV) of oxyglibin were also administered. Misoprostol (5 μg/kg [2.3 μg/lb], PO, q 8 h) was given because of the ongoing hematemesis, and ondansetron (0.15 mg/kg [0.07 mg/lb], IV, q 6 h) was administered because of the persistent nausea and vomiting. Tramadol (2.5 mg/kg, PO, q 8 to 12 h) was administered for its analgesic effects, and all other medications were continued as before.

On the eighth day of hospitalization, there was evidence of pulmonary edema or hemorrhage, with crackles in all lung fields. The PCV had decreased from 32% to 13% over the previous 2 days, and the dog had signs of increasing respiratory compromise. Therefore, supplemental oxygen was provided. Respiratory and cardiac arrest occurred, and attempts at cardiopulmonary resuscitation were unsuccessful.

The clinical course for dog 6 was similar to, but less severe than, the clinical course for dog 5. Proteinuria was less severe, oliguric renal failure did not develop, and there was no evidence of coagulopathy. However, 28 days after administration of human albumin solution, bacterial culture of blood samples from dog 6 yielded multiple colonies of multidrug-resistant Escherichia coli. These findings were accompanied by a severe worsening of the dog’s condition, which culminated in septic shock, fulminant noncardiogenic pulmonary edema (presumed to be secondary to acute respiratory distress syndrome), and respiratory and cardiac arrest. Attempts at cardiopulmonary resuscitation were unsuccessful.

Histologic examination of necropsy specimens from dogs 5 and 6 revealed vascular changes ranging from mild fibrinoid degeneration to severe necrosis and inflammation that obliterated the vessel wall. In the more mildly affected vessels, small accumulations of fibrin were visible within the tunica media. Occasionally, this fibrin was accompanied by small numbers of mononuclear inflammatory cells. In more severely affected vessels, the fibrinoid degeneration often involved all layers of the vessel wall with no evidence of morphologically normal tissue. In addition to the fibrinoid degeneration and necrosis, the most severely affected vessels also had large numbers of neutrophils, lymphocytes, and macrophages that effaced the vessel walls and infiltrated adjacent tissues. These vessels were frequently surrounded by severe hemorrhage and edema. Many of the vessels with evidence of fibrinoid degeneration and necrosis, as well as many vessels unaffected by these changes, contained large fibrin thrombi. In the kidneys, these changes were accompanied by a moderate to severe increase in the cellularity of glomerular tufts and moderate thickening of the mesangium (membranoproliferative glomerulonephritis). The most severe lesions were located in the heart, liver, kidneys, stomach, small intestine, and urinary bladder. Less severe vascular lesions were observed in the meninges, brain, tongue, tonsils, lungs, spleen, adrenal glands, pancreas, esophagus, large intestine, skeletal muscle, and skin.

Specimens from dogs 5 and 6 were also submitted for electron microscopy. These specimens were fixed in 3% phosphate-buffered glutaraldehyde (pH, 7) for 24 hours, postfixed for 1 hour in 1% aqueous osmium tetroxide, and embedded in resin. Sections were cut at a thickness of 1 μm, stained with toluidine blue, and examined at a magnification of 100X to identify areas with glomeruli. Blocks identified as containing areas with glomeruli were used to obtain thin sections for electron microscopy. Three grids each containing 3 thin sections were prepared from each block. Thin sections were stained with uranyl acetate and lead citrate and examined.

Transmission electron microscopy revealed that the basement membrane of the glomeruli was diffusely expanded by multifocal, heterogeneous, electron-dense material (arrowheads) that extends into the urinary space (U). Portions of a mesangial cell (M) can be seen. Bar = 1,000 nm.
thickened. Podocytes lining the basement membrane and urinary space were effaced or fused. The lamina rara externa or the subepithelial surface was irregularly expanded by large, heterogeneous, electron-dense deposits (Figure 1). Findings were consistent with antigen-antibody deposition.

An ELISA was developed to detect anti-human albumin antibodies in canine serum. Plates were coated with 25% human albumin solution, and serum samples were added to the wells, followed by anti-canine IgG antibody conjugated with horseradish peroxidase. Checkerboard titrations were performed to optimize the assay. Two experimental dogs were hyperimmunized with 25% human albumin solution to produce canine anti-human albumin antibody for the assay.

In all 6 dogs, development of antibodies against human albumin was confirmed with the ELISA. All dogs had high concentrations of anti-human albumin antibodies shortly after administration of human albumin solution, with antibody concentration peaking 2 to 4 weeks after human albumin administration (Figure 2). Dog 1 was the only dog that had a high anti-human albumin antibody concentration prior to administration of human albumin solution and was also the only dog that developed signs of an immediate hypersensitivity reaction. Whether this dog had preexisting human albumin-specific IgE or an acute proinflammatory cytokine response was not determined.

To confirm reactivity with human albumin and to determine whether dogs might be responding to a contaminating plasma protein, serum samples from the 6 dogs were examined by means of immunoblotting (Figure 3). Proteins in the commercial product were mixed with sample buffer, separated by means of gel electrophoresis, and electrothermotically transferred to nitrocellulose membranes. Membranes were probed with serum samples, and bound antibody was detected with secondary anti-canine IgG antibody by means of chemiluminescence. Results of immunoblotting indicated that all 6 dogs developed anti-human albumin antibodies, as evidenced by reactivity with a protein migrating at 66 kd. However, serum from all 6 dogs also reacted with other minor bands in the human albumin solution.

To rule out the possibility that the particular lot of human albumin solution used in these 6 dogs was contaminated, samples were submitted for aerobic and anaerobic bacterial culture. However, no growth was obtained.

A sample from this lot and a sample from a separate lot of human albumin solution were submitted for various protein identification techniques to identify any possible contaminants. Both samples were digested with trypsin, and the resulting peptides were separated by means of
In reviewing the records of the Washington State University Veterinary Teaching Hospital from 2001 through 2005, we identified 92 patients (88 dogs, 2 cats, 1 calf, and 1 ferret) that were given human albumin solution. Five of the 88 (5.7%) dogs developed acute hypersensitivity reactions, including 2 dogs that developed a fever, 2 dogs that developed facial edema, and 1 dog that developed facial edema and urticaria. All reactions resolved after administration of human albumin solution was discontinued and diphenhydramine was administered. None of the 88 dogs had any evidence of delayed or type III hypersensitivity reactions following treatment with human albumin solution.

In 2 reports, of healthy dogs with normal serum albumin concentrations given human or bovine albumin solution, mild to severe adverse reactions were documented. In one of these reports, 4 of 9 dogs developed severe adverse reactions following administration of human albumin solution, including anaphylactic shock in 1 dog given 1 mL of 25% human albumin solution IV. The dog recovered with treatment but developed severe facial edema and urticaria 6 days later. A second dog developed severe facial, limb, and ventral edema 7 days after administration of human albumin solution (30 g administered at a rate of 0.5 mL/kg/h). In that report, 2 dogs that did not have any adverse reactions following an initial infusion of human albumin solution were given a second dose 5 weeks later. Both of these dogs developed anaphylactic shock after IV infusion of only 0.2 to 0.3 mL of human albumin solution. Both dogs recovered, and neither developed edema or urticaria. In the other report, 10 healthy mature dogs were given bovine serum albumin solution. One dog developed an acute hypersensitivity reaction consisting of mild urticaria and pruritus, but the 9 remaining dogs did not have any immediate reactions. Two dogs received a second dose of bovine serum albumin solution 14 days later. One developed a mild immediate reaction similar to that occurring after administration of the first dose of bovine serum albumin solution, and the other developed a severe anaphylactic reaction. Five dogs that had no acute reaction to the administration of bovine serum albumin solution and the 1 dog with the mild acute reaction following the second administration of the product developed mild to severe generalized type III hypersensitivity reactions a mean of 15 days after bovine serum albumin solution administration. However, all dogs that developed a reaction recovered. Three dogs did not have any adverse reactions to bovine serum albumin solution administration. The conclusion of these investigators was that prior natural exposure to bovine serum albumin must have occurred and that, because of the high complication rate, bovine serum albumin solution is not a suitable therapeutic option for use in dogs.

The reactions seen in the 6 dogs described in the present report were consistent with type III hypersensitivity reactions or immune complex-mediated hypersensitivity. These types of reactions typically begin 3 to 8 hours after exposure to an excess of foreign antigen; however, lesions may not be apparent until several days later. Lesions of cutaneous neutrophilic vasculitis found in biopsy and necropsy samples from 2 of these dogs were consistent with the Arthrus reaction and are highly suggestive of an immune complex disorder.
Systemic signs of a type III hypersensitivity reaction, or serum sickness, developed in these 6 dogs between 5 and 13 days (median, 12 days) after IV administration of human albumin solution. Serum sickness is defined as a constellation of clinical signs including fever, lymphadenopathy, generalized urticaria, and polyarthritids resulting from immune complex deposition occurring when there is antigen excess. This syndrome was historically identified when human patients were given large doses of foreign serum (eg, horse antitoxin) by injection, with many patients developing signs of the syndrome approximately 8 days following injection. The pathogenesis involves synthesis of antibodies that bind to the antigens, forming soluble immune complexes in the presence of moderate to gross antigen excess. These immune complexes precipitate in the venules, most commonly in venules of the skin, joints, kidneys, and heart. An inflammatory cascade ensues as complement is bound and anaphylatoxin is generated, leading to mast cell degranulation. Histamine released from mast cell granules causes increased vascular permeability, local platelet aggregation, and vasoactive amine release from activated platelets. The cascade of events leads to basement membrane damage with resultant vasculitic skin lesions, immune-mediated polyarthritis, proteinuria, and myocardial lesions with potentially secondary arrhythmias.

The reactions seen in the 6 healthy dogs described in the present report have not, to our knowledge, been identified following administration of human albumin solution to hypoalbuminemic dogs. All 6 dogs received human albumin solution from the same lot, and according to the manufacturer, there have been no reports of human patients developing adverse reactions following administration of human albumin solution from this lot. To rule out the possibility that reactions in these dogs were a result of contaminants, bacterial culture and molecular analysis were performed. No bacterial contaminants were identified, and the only difference between this lot of human albumin solution and samples from a second lot was a trace amount of lactoferrin. The possibility remains that human albumin solution used in these dogs may have been contaminated with something that was not identified with our analysis; however, this seems unlikely, given that no adverse reactions were reported in human patients that received doses of human albumin solution from the same lot.

There is incomplete homology between the human and canine albumin molecules (79.3% homology). Therefore, the human albumin molecule is potentially antigenic when administered to dogs. In the clinical setting, dogs receiving human albumin solution are hypoalbuminemic and generally have immunosuppression as a result of the same process that caused the hypoalbuminemia. Albumin is responsible for binding free fatty acids, free radical species, bilirubin, and other toxins in circulation, thus mediating inflammation and secondary multisystemic tissue damage. These immunomodulatory effects of albumin are lost in hypoalbuminemic patients. Therefore, it may be hypothesized that the immunocompetence of healthy dogs described in the present report predisposed them to develop type III hypersensitivity reactions. Alternately, it may be hypothesized that following administration of human albumin solution to normoalbuminemic dogs, the supraphysiologic dose of albumin overrode the body’s ability to tolerate the foreign protein through some unknown mechanism. Rapid administration of human albumin solution may adversely affect a patient’s ability to compensate for the increase in serum albumin concentration. Administration of human albumin solution over 12 to 24 hours may result in fewer immunologically mediated adverse effects. However, 25% human albumin solution contains no preservatives, and the manufacturer warns against administration of the solution if the seal has been punctured for ≥4 hours because of the potential for bacterial contamination. In addition, the dose of human albumin solution that is administered may affect development of adverse reactions, and it is possible that a lower dose of human albumin solution may be better tolerated than a higher dose. However, a previous study found that even small volumes of human albumin solution could cause severe immediate and delayed reactions. Finally, it is possible that some dogs given human albumin solution because of hypoalbuminemia may not have survived long enough for signs of a type III hypersensitivity reaction to develop. However, at least some of these dogs have survived, and to our knowledge, there have not been any reports of dogs that developed such reactions after administration of human albumin solution for treatment of hypoalbuminemia. Regardless, it was noteworthy that all 6 healthy dogs described in the present report developed signs of serum sickness following administration of human albumin solution. Differences in the severity of signs among dogs may be attributable to inherent differences in their immune systems; however, all 6 had reactions consistent with a type III hypersensitivity reaction.

Findings in the dogs described in the present report suggest that further investigation is required on the use of human albumin solution in dogs, but also raise the question of whether healthy dogs are an appropriate model for critically ill dogs that would receive human albumin solution in clinical practice. In particular, our findings suggest that the immunologic consequences may be different when human albumin solution is administered to healthy versus critically ill dogs and raise concerns about the possible consequences of acute hyperalbuminemia. Regardless, our findings suggest that caution is warranted when administering human albumin solution to dogs, even if human albumin has never been administered previously, and that the potential benefits of human albumin solution should be weighed against the risks of adverse reactions. In general, it appears that human albumin solution could be administered to hypoalbuminemic dogs if other forms of treatment have failed and should not be administered to normoalbuminemic dogs.

a. Plasbumin-25, Bayer Corp, Elkart, Ind.
b. Cibacron Blue, Pierce Biotechnology Inc, Rockford, Ill.
transfusions in a veterinary critical care facility: 5 cases (abstr).

References

Selected abstract for JAVMA readers from the American Journal of Veterinary Research

Evaluation of adverse effects of long-term oral administration of carprofen, etodolac, flunixin meglumine, ketoprofen, and meloxicam in dogs
Stelio P.L. Luna et al.

Objective—To evaluate adverse effects of long-term oral administration of carprofen, etodolac, flunixin meglumine, ketoprofen, and meloxicam in dogs.

Animals—36 adult dogs.

Procedures—Values for CBC, urinalysis, serum biochemical urinalyses, and occult blood in feces were investigated before and 7, 30, 60, and 90 days after daily oral administration (n = 6 dogs/group) of lactose (1 mg/kg, control treatment), etodolac (15 mg/kg), meloxicam (0.1 mg/kg), carprofen (4 mg/kg), ketoprofen (2 mg/kg for 4 days, followed by 1 mg/kg daily thereafter), or flunixin (1 mg/kg for 3 days, with 4-day intervals). Gastroscopy was performed before and after the end of treatment.

Results—For serum y-glutamyltransferase, values were significantly increased at day 30 in dogs treated with lactose, etodolac, and meloxicam within groups. Bleeding time was significantly increased in dogs treated with carprofen at 30 and 90 days, compared with baseline. At 7 days, bleeding time was significantly longer in dogs treated with meloxicam, ketoprofen, and flunixin, compared with control dogs. Clotting time increased significantly in all groups except those treated with etodolac. At day 90, clotting time was significantly shorter in flunixin-treated dogs, compared with lactose-treated dogs. Gastric lesions were detected in all dogs treated with etodolac, ketoprofen, and flunixin, and 1 of 6 treated with carprofen.

Conclusions and Clinical Relevance—Carprofen induced the lowest frequency of gastrointestinal adverse effects, followed by meloxicam. Monitoring for adverse effects should be considered when nonsteroidal anti-inflammatory drugs are used to treat dogs with chronic pain. (Am J Vet Res 2007;68:258–264)