Intravascular hemolysis associated with severe cutaneous burn injuries in five horses

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- Hemolysis is a potential sequela of severe cutaneous burn injury in horses.
- Considerable hemolysis may predispose horses with cutaneous burns to development of pigment nephropathy and renal failure.

Five horses (4 Quarter Horses and 1 Arabian; age range, 5 to 11 years) were evaluated at the **Texas Veterinary Medical Center (TVMC)** of Texas A&M University because of severe cutaneous burn injuries and suspected smoke inhalation following an overnight fire of the barn in which the horses were housed. The 30-stall wood-frame construction barn with open rafters was quickly and completely destroyed by the fire. Of the 27 horses housed in the barn, 16 died in the fire and 6 escaped serious injury; the remaining 5 horses required veterinary medical treatment. Time intervals from the fire to the time of admission ranged from 4 to 14 hours. Of the 5 horses requiring treatment, horses 2, 3, and 5 had received 500 mg of flunixin meglumine IV at the scene of the fire.

At admission, blood samples were collected from each horse; gross hemolysis was detected in plasma obtained from all 5 samples, and microscopic examination of blood smear preparations revealed signs of physical or oxidative injury of RBCs. Morphologic changes in the erythrocytes included the presence of eccentrocytes, spherocytoid cells (ie, RBCs resembling spherocytes), formation of Heinz-body–like structures, and membrane blebbing with fragmentation.

The most severely affected horse admitted to the TVMC (horse 1) was a 6-year-old Quarter Horse gelding that reportedly broke out of its stall and ran from the burning barn. According to an observer, the horse was engulfed in flames when it exited the barn. The horse could not be located until 14 hours later, at which time it was immediately transported to the hospital. A physical examination was performed at the time of admission; rectal temperature was 39.6°C (103.4°F), heart rate was 72 beats/min, and respiratory rate was 42 breaths/min. The horse's breathing was shallow, and on thoracic auscultation, breath sounds were quiet. The horse was profoundly weak and nearly collapsed several times during the initial evaluation. Severe partial and full-thickness burns were estimated to involve 70% of the horse's body surface area, including the entire head, eyelashes, corneas, ears, neck, dorsum, ventrum, and distal portions of the limbs. The horse was dehydrated (PCV, 60% [reference range, 32% to 53%]; plasma total solids concentration, 11.0 g/dL [reference range, 5.3 to 7.3 g/dL]) and azotemic (serum creatinine concentration, 6.6 mg/dL [reference range, 1.1 to 2.0 mg/dL]). The horse did not void urine during hospitalization. A plasma sample from the horse was markedly hemolyzed, and a microscopic examination of a blood smear revealed many erythrocyte fragments, a moderate number of spherocytoid cells, and a moderate degree of eccentrocytosis. Initial resuscitative treatments included IV administration of saline (7.0% NaCl) solution (4 mL/kg [1.8 mL/lb]) and a 10-L bolus of lactated Ringer's solution; the horse received oxygen (10 L/min) intranasally. Butorphanol was administered for pain management; 15 mg was given IV initially followed by a continuous IV infusion (10.5 mg/h). Because of the grave prognosis for the horse's survival, the owner requested euthanasia; a necropsy was not performed.

An 11-year-old Arabian gelding (horse 2) was admitted to the TVMC approximately 7 hours after the fire. On physical examination, the horse had grossly visible burns with associated generalized subcutaneous edema over its head (including muzzle), neck, dorsal and lateral aspects of the thorax, and rump. The total body surface area burned was estimated to be 50%. Rectal temperature was 37.5°C (99.5°F), and heart rate was 54 beats/min. The respiratory rate was 24 breaths/min, and mild upper respiratory stridor was audible; however, no abnormalities were detected via thoracic auscultation. The right periocular region was markedly swollen, and the muzzle was deviated to the left (indicative of facial nerve paralysis). Fluorescein stain was applied to both eyes to evaluate corneal integrity, and there was no uptake of stain to suggest corneal damage. Mucous membranes were slightly tacky, and gastrointestinal tract sounds were decreased on auscultation of all abdominal quadrants. Laboratory analyses revealed that PCV was 42% and plasma total solids concentration was 6.8 g/dL; the plasma was moderately hemolyzed.

Examination of a blood smear revealed morphologic abnormalities among erythrocytes that were similar in type and magnitude to those identified in horse 1. Osmotic fragility of erythrocytes in horse 2 was evaluated to determine whether those cells were abnormally susceptible to osmotic stress, which is a feature of Heinz body anemia and spherocytosis. This was accomplished by exposing samples of erythrocytes to

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Presented in part at the 50th Annual Convention of the American Association of Equine Practitioners, Denver, December 2004.

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graded concentrations of increasingly hypotonic saline solutions (0.9% to 0%) and then measuring the hemoglobin concentration of the supernatant as an indication of hemolysis.[1,2] Compared with results of the evaluation of erythrocytes from a clinically normal horse, the osmotic fragility of erythrocytes from horse 2 was increased, indicating that the erythrocytes of horse 2 were abnormally vulnerable to damage induced by osmotic stress. Results of venous blood gas analysis were within reference limits; attempts to obtain an arterial blood sample were unsuccessful because of the degree of subcutaneous edema. No abnormalities were detected via serum biochemical analyses other than high creatinine concentration (2.4 mg/dL). Findings of thoracic radiography were unremarkable, and radiography of the head revealed no bony abnormalities. The horse had signs of slight depression but ate, drank water, and produced manure normally during the first 12 hours of hospitalization.

During the 24 hours following admission, the horse produced only a scant volume of dark brown urine. During this period, the subcutaneous edema of the head and distal portion of the limbs progressed dramatically, causing the skin to split and ooze serum at multiple sites. Edema of the nares and nasal mucosa also progressed and necessitated a tracheostomy to bypass the resulting airway occlusion. After placement of the tracheostomy tube, the horse coughed periodically and produced brown viscous mucoid material through the tube. Thoracic radiography was performed and revealed low-grade pneumonitis consistent with mild smoke inhalation.

Despite administration of lactated Ringer's solution (2.5 mL/kg/h [1.14 mL/lb/h], IV) and furosemide (1 mg/kg [0.45 mg/lb], IV, q 4 h), the horse became increasingly hemoconcentrated (PCV, 51%), anuric, and markedly azotemic (serum creatinine concentration, 8.3 mg/dL). After the horse had been hospitalized for 34 hours, the owner requested euthanasia because of deterioration of the horse's clinical condition; a necropsy was performed. In addition to cutaneous burn injuries and severe edema of affected skin and nasal mucosa, the necropsy revealed severe hemoglobinuric nephrosis characterized by tubular occlusion with proteinaceous hemoglobin casts. An ammonium sulfate precipitation test performed on a sample of the urine in the bladder yielded positive results for hemoglobin and negative results for myoglobin. Further findings included mild pulmonary changes characterized by multifocal alveolar hemorrhages and accumulations of necrotic debris in the trachea and bronchi; findings also included splenic hemosiderosis and erythrophagocytosis.

Horse 3 was a 9-year-old Quarter Horse that was examined approximately 5 hours after sustaining burn injuries. The distribution of the cutaneous burns was similar to that of horse 2, but the injuries were less extensive and involved approximately 25% of the total body surface area. Nonetheless, horse 3 had marked signs of depression, inappetence, and no interest in drinking; it became anxious and somewhat aggressive when handled. At this initial evaluation, heart rate was 52 beats/min, respiratory rate was 16 breaths/min, and rectal temperature was 39.1°C (102.5°F). Auscultation of the thorax and serial radiographs of the thorax obtained during the first 3 days of hospitalization revealed no abnormal findings. Borborygmi were reduced in all abdominal quadrants. The horse had moderate hemoconcentration (PCV, 53%; plasma total solids concentration, 6.6 g/dL) and azotemia (serum creatinine concentration, 3.2 mg/dL). Moderate hemolysis of the plasma was detected, and RBC morphologic changes consistent with physical and oxidative damage were observed via microscopic examination of a blood smear. Osmotic fragility of horse 3's erythrocytes was evaluated and determined to be increased, compared with that of erythrocytes of a clinically normal horse. Microscopically, the extent of the erythrocyte abnormalities was less than that detected in the first 2 horses, with only rare spherocytoid erythrocytes and RBC fragments detected.

Horse 3 failed to produce urine during the first 17 hours of hospitalization, despite IV administration of a 10-L bolus of lactated Ringer's solution followed by continuous infusion of fluids (6 mL/kg/h [2.73 mL/lb/h]) and treatment with furosemide (1 mg/kg, IV, q 6 h). During the next 3 days, the horse became progressively azotemic and serum creatinine concentration reached 13.5 mg/dL on day 3 of hospitalization. Polyuria was achieved after 32 hours of aggressive fluid and diuretic treatments, and from day 3 of hospitalization, the serum creatinine concentration steadily decreased to 5.0 mg/dL. Serial urinalyses revealed hyposthenuria, and fine granular casts were detected cytologically in urine samples on days 2 to 7. Results of an ammonium sulfate precipitation test performed on a sample of the urine confirmed the presence of hemoglobin in horse 3's urine; however, no myoglobin was identified. Gross pigmenturia was not observed in this horse at any time during hospitalization. By day 3 of hospitalization, the erythrocyte morphologic changes had resolved and erythrocyte osmotic fragility had steadily improved and returned to within reference limits. During this time, the horse's attitude and appetite also steadily improved. On day 7 of hospitalization, the horse developed severe acute laminitis and was euthanatized at the request of the owner. A necropsy revealed chronic nephrosis with tubular degenerative change and regeneration, and there were granular pigmented casts within the renal tubules. Other postmortem findings included splenic hemosiderosis and full-thickness cutaneous burns with marked subcutaneous edema; the lungs were grossly and histologically normal.

Horses 4 and 5 were 5-year-old and 10-year-old Quarter Horse geldings, respectively. The horses were admitted to the hospital approximately 8 (horse 4) and 5.5 (horse 5) hours after sustaining burn injuries. They both had burn distributions similar to that of horse 3, with cutaneous burns to the head, neck, and dorsum; burn injuries extended over 25% and 35% of the total body surfaces of horses 4 and 5, respectively. Each horse was mildly febrile and mildly azotemic and had moderate hemoconcentration. The use of a rebreathing bag to enhance thoracic auscultation of horse 4 elicited a cough, and black sputum was produced. In horse

5, findings of thoracic radiography indicated no abnormalities; thoracic radiographs were not obtained for horse 4. Fluorescein staining of the corneas of horse 5 revealed a superficial ulcer of the right cornea, which resolved after several days of topical treatment with triple antimicrobial ointment.

Moderate hemolysis was detected in samples of plasma from both horses. Evaluation of smears of blood from horse 5 revealed morphologic changes among erythrocytes that were similar to those detected in blood smears from horses 1, 2, and 3; the severity of those changes was comparable to that detected in horse 3. Compared with findings in horses 1, 2, 3, and 5, erythrocyte abnormalities were less severe in horse 4; only rare RBC fragments were observed, and no spherocytoid erythrocytes were evident. Both horses were bright and had a normal attitude and appetite. Within 6 hours of admission, horse 5 developed severe pigmenturia consistent with hemoglobinuria, which persisted for 3 days. Analysis to determine the source of the pigment was not pursued. Horse 5 became mildly azotemic (serum creatinine concentration, 3.7 mg/dL); after IV administration of lactated Ringer's solution (4 mL/kg/h) and furosemide (1 mg/kg, q 4 h) for several days, the azotemia resolved. Compared with findings in erythrocytes of a clinically normal horse, the osmotic fragility of horse 5's erythrocytes was increased. Horses 4 and 5 responded well to treatment, and they were eventually discharged from the hospital. At 11 months after the original injuries, treatment of extensive epithelial loss secondary to the original burns was still ongoing in these horses.

For the 5 horses of this report, the overall goals of treatment included wound care, correction of dehydration and provision of diuresis, control of inflammation, pain management, and prophylaxis against sepsis. Certain procedures and treatments were common to all patients. On arrival at the intensive care unit, each horse was bathed with cool water to wash away burned hair and ash and decrease surface temperature. Silver sulfadiazine was applied topically to all raw, burned areas at 6- to 12-hour intervals. Because of the extensive burns of the horses' muzzles, smoke inhalation was suspected. Intranasal administration of oxygen was attempted in every horse on admission, but was only tolerated by horse 1. Generally, the placement of cannulae for intranasal administration of oxygen appeared to cause intense irritation of the burned portion of the nares and nasal mucosa; either horse 1 was obtunded to the point of diminished responsiveness or the injuries to the nasal mucosa were severe enough to cause denervation. Despite facial burn patterns suggestive of smoke inhalation, only horses 2 and 4 had clinical or radiographic signs of pulmonary compromise secondary to smoke inhalation. These were transient in horse 4; horse 2 was euthanatized soon after signs of pulmonary damage became noticeable, and necropsy revealed mild pulmonary changes. Having visited the site of the fire, we suspect that the open beam construction of the barn minimized trapping of smoke and limited the exposure of the horses to smoke and caustic fumes.

Fluid therapy was administered on the basis of each horse's needs and willingness to drink water. Horse 1 was aggressively treated with fluids IV and received hypertonic saline solution and a 10-L bolus of lactated Ringer's solution; horse 3 also received a 10-L bolus of fluid IV on admission. Determination of the rate and volume of fluid administration that would meet metabolic demands yet not potentiate development of peripheral and pulmonary edema was extremely challenging, especially in horse 2. Diuresis was not attained in this horse, despite fluid and diuretic treatments. Nonetheless, marked subcutaneous interstitial edema progressed rapidly, leading to occlusion of portions of the respiratory tract.

Anti-inflammatory agents administered to the 5 horses included flunixin meglumine (1.1 mg/kg [0.5 mg/lb], IV, q 12 h) and dexamethasone (0.1 mg/kg [0.045 mg/lb], IV, q 24 h) initially; dexamethasone was discontinued after the second day of hospitalization. The administration of corticosteroids to burn patients is controversial[3,4]; they were used in these horses to decrease inflammation and edema and for membrane stabilization. Flunixin meglumine is potentially nephrotoxic, but administration of this drug was deemed necessary to alleviate inflammation and signs of pain. Horses 2 and 3 additionally received **dimethyl sulfoxide** (**DMSO**; 1 g/kg [0.45 g/lb; administered as a less than 10% solution], IV, q 24 h) because of its anti-inflammatory and diuretic effects. At concentrations of 20% or greater, DMSO can cause hemolysis[5]; a low concentration was used in these horses, and DMSO was not administered until after RBC morphology had been evaluated. Enrofloxacin (5.0 mg/kg [2.3 mg/lb], IV, q 24 h) and doxycycline (10 mg/kg [4.5 mg/lb], PO, q 12 h) were administered for prophylaxis against sepsis. These drugs were chosen because of their combined broad antimicrobial actions and low potential for renal toxic effects[3]; the latter was important because factors contributing to renal function compromise were already present in these horses. The use of antimicrobials in humans with burn injuries is controversial because of concerns about the development of resistant strains of bacteria. However, unlike human burn patients, horses are housed in an environment rich in organic matter and contaminants, and sterile bandaging of large parts of their bodies is not feasible. Therefore, we chose to administer antimicrobials to the horses as a prophylactic measure against development of burn-associated sepsis. Pain management was achieved via continuous rate infusions of butorphanol (0.025 mg/kg/h [0.011 mg/lb/h]). For horses 4 and 5, butorphanol was discontinued on day 3 of hospitalization and a continuous rate IV infusion of ketamine (0.4 mg/kg/h [0.18 mg/lb/h]) was started. The other treatments for each horse have been described.

Severe burn injury has been associated with hematologic changes in humans, including hemoconcentration, decreased blood volume due to exudation of plasma, decreased erythrocyte deformability, and intravascular hemolysis. These changes and associated severe complications have long been recognized and have lead to the development of murine, canine, and lagomorphic experimental models of this phenomenon.[1,2,6-10] Erythrocyte morphologic changes associated with severe burns in human patients and laboratory animals are consistent

with oxidative damage to the cell membrane and include eccentrocytosis, spherocytosis, fragmentation, and increased osmotic fragility.[2,7,8,10] Membrane fragmentation apparently contributes to renal failure in humans.[7] The risk of renal failure is increased in dogs with hemoconcentration and dogs in which catecholamine response leads to a redistribution of blood flow.[11] All 5 of the horses reported here had some degree of gross hemolysis at their initial examination and had morphologic evidence of oxidative damage to RBCs. These morphologic changes resolved within the first 2 to 3 days of hospitalization in all horses that survived. In addition, compared with findings in erythrocytes of clinically normal horses, osmotic fragility of erythrocytes in horses 2, 3, and 5 was increased; this change was coincident with the presence of abnormal erythrocytes. Differential diagnoses for hemolysis secondary to oxidative damage to erythrocytes in horses include red maple leaf, wild onion, or phenothiazine toxicosis. According to farm personnel, these horses had no exposure to these substances and were in apparent good health prior to the fire.

Results of serial urinalyses performed for horse 3 and postmortem urinalysis performed for horse 2 indicated that there was hemoglobin in the urine; no myoglobin was detected in any urine sample. Horse 5 developed severe pigmenturia, which was assumed to be hemoglobinuria, but analysis to determine the source of the pigment was not pursued. Necropsy findings of horses 2 and 3 were consistent with severe hemoglobin pigment nephropathy. In horse 2, acute renal changes included granular proteinaceous pigment casts, moderate nephrosis, and nearly complete obstruction of the nephrons by hemoglobin casts. Renal lesions in horse 3 were associated with more chronic changes, including tubular degeneration and regeneration, granular pigmented casts, and lymphoplasmacytic interstitial nephritis.

The exact mechanisms of RBC damage and destruction following burn injury are not fully understood. The degree of hemolysis appears to be proportional to the severity and extent of cutaneous burns, and hemolysis has been reported in humans and rats with at least 15% of their total body surface area affected by second-degree burns.[1,8] Anemia and renal failure in human burn patients are in part attributed to burn-related hemolysis.[7,8] None of the horses of this report developed anemia during hospitalization, although hemoconcentration may have masked anemia in some of them. In these horses, the severity of hemolysis and RBC morphologic abnormalities correlated with the severity of burns and systemic illness associated with burn injury. In vitro, direct thermal injury of human RBCs results in loss of biconcavity and the formation of surface buds.[2] Results of several studies[1,7-10] also suggest that the release of reactive oxygen species by complement-activated neutrophils is involved in the pathogenesis of hemolysis associated with burn injuries. Complement activation may persist for several days after burn injury, markedly decreasing the survival time of transfused RBCs from clinically normal donors. In contrast, in studies[7,8] involving labeled RBCs, transfusion of erythrocytes from burned to clinically normal human patients resulted in normal survivability of transfused cells. This finding supports the suggestion that intravascular hemolysis in severely burned individuals is induced by circulating inflammatory mediators and not direct thermal injury to erythrocytes.

Hatherill et al[1] investigated the role of complement activation in burn trauma-associated hemolysis in rats and found that maximal hemolysis occurred during the 15-minute period immediately after the induction of burn injury. Complement binding to erythrocytes was not increased during that period. In that study,[1] depletion of complement, depletion of neutrophils, or pretreatment with oxygen-radical–attenuating substances such as catalase, superoxide dismutase, DMSO, or dimethyl thiourea substantially attenuated osmotic fragility and hemo-lysis secondary to burn trauma. These authors concluded that hemolysis occurs secondary to damage to erythrocyte membranes caused by reactive oxygen species; the erythrocytes are exposed to the reactive oxygen species through chance interactions with neutrophils that have been activated by complement.

A similar study by Oldham et al[9] revealed that chemotaxis mediated by complement protein 5_a was increased at the site of experimentally induced burn injury in rats. This chemotaxis occurred even in neutrophil-depleted animals. Inhibition of xanthine oxidase with allopurinol and lodoxamide abolished increased complement-mediated chemotactic activity. This suggests that the initial hydroxyl radical surge, which activates complement, is produced by nonphagocytic cells and is xanthine oxidase dependent. In this study,[9] as in the study by Hatherill et al,[1] hydroxyl radical-scavenging drugs, including DMSO, were found to prevent activation of complement and attenuated neutrophil chemotaxis and RBC lysis. Other authors have reported[10] that superoxide dismutase administered 30 minutes before or directly after burn injury reduced hemolysis and associated renal failure in rats. Two of the horses reported here received DMSO IV; any specific clinical benefit to those horses is undetermined, as both had developed hemolysis prior to administration of DMSO.

The structural changes to the erythrocyte membrane following burn injury have been investigated; after severe burn injuries in rats,[8] osmotic fragility increases and membrane deformability decreases, resulting in accelerated destruction of RBCs. In that study, osmotic fragility was determined to be maximally increased 4 to 8 hours after cutaneous burn injury. By contrast, the maximum decrease in membrane deformability occurs within 1 hour of burn injury but persists for at least 8 hours.[8] Reported morphologic changes in erythrocytes after thermal injury include crenation, eccentrocytosis, spherocytosis, bud formation, and fragmentation.[1,7-9] The RBCs of the horses of this report had these abnormalities to various degrees, with the most severe changes to erythrocytes evident in the 2 horses with the most extensive burn injuries. Hemoconcentration, hemoglobinemia, and hemoglobinuria are also typical clinical features in burn patients; in experimental studies[2,8] in rats, these clinical signs are transient in individuals that receive prompt, aggressive fluid resuscitation, provided that their renal function is normal.

The efficacy of treatment for burn injury–associated hemolysis is related to the time frame in which it is administered. Alterations in erythrocyte membranes and hemolysis peak at approximately 15 minutes after burn injury in rats.[1,10] Experimentally, successful attenuation of the inflammatory and hemodynamic effects of burn injury is achieved when interventions are applied shortly before or immediately after induction of burn trauma. In clinical reports[12] of burn injury in human patients, the importance of early intervention is emphasized and the development of burn-associated renal failure has been positively correlated to length of time between injury and hospital admission. Guidelines for emergency treatment of human burn patients instruct that fluid therapy should be initiated prior to transport in patients with burns affecting > 25% total body surface area or in any burn patient for whom the interval from injury to hospital admission is anticipated to exceed 1 hour.[13] Among the horses of this report, the shortest interval from injury to admission was 4 hours; all horses had burn injuries to more than 25% of their body surface areas.

The findings regarding time frames of successful treatment of burn-associated injuries in rats and humans may have relevance to the emergency management of severe burn injury in horses. Many barn fires occur in the middle of the night, and there may be delays in contacting owners and the subsequent transportation of horses to veterinary clinics for treatment. In the situation reported here, trailer movement from the property was prevented by the presence of emergency vehicles on the access road, and horses running from the scene of the fire were missing for extended periods, further delaying their transport to a care facility. Experimental findings in animals and the experience of physicians working in human burn units suggest that initial on-site treatment of horses with cutaneous burns should include administration of IV fluids as soon after the time of injury as possible.[2,12,13] Fluid volume determination can be difficult in these patients because fluid overload can result in serious pulmonary complications.[13] The Parkland formula, which is used commonly in humans, incorporates the extent of epithelial damage in estimating fluid requirements (4 mL/kg/percentage of body surface area burned)[13] but may be difficult to apply in horses because the extent of burn injury is often not apparent for several days. However, the ability to estimate fluid requirements and administer them at the site of the fire may be lifesaving. This intervention is intended to correct hemoconcentration, improve rheologic properties of blood, and provide adequate perfusion to the kidneys and other organs.[2,7,8,11] If administered promptly following burn injury, DMSO (1 g/kg, IV, administered as a < 10% solution) or xanthine oxidase inhibitors (eg, allopurinol or lodoxamide) may also yield therapeutic benefit in preventing or reducing erythrocyte fragmentation and hemolysis associated with burn injury. However, clinical reports supporting the use of these drugs for this purpose are lacking. Evidence of gross hemolysis or hemoglobinuria in a horse that has suffered cutaneous burns should alert the clinician to the severity of the burn trauma and the potential complications thereof.

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