Histochemical Changes of Skeletal Muscle in Equine Acute Rhabdomyolysis

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Abstract. In two mixed breed horses that died due to acute rhabdomyolysis, were realized histopathology and histochemical exams. There were examined the following muscles: mean gluteus, semitendinosus and semimembranosus. Histopathology exam revealed some specific lesions for acute rhabdomyolysis, such as interfibrillar edema, granular and vacuolar degeneration and Zenker myofiber necrosis. Histochemical exam noticed in affected myofibers normal glycogen and amylase-resistant polysaccharide complex. Myofibers lesions degree was more severe in muscle cells that were enriched in PAS positive amylase-resistant polysaccharide material. A certain cause of acute rhabdomyolysis in both horses was polysaccharide storage myopathy in which the muscular presence of amylase-resistant polysaccharide complex is the pathognomonic finding.

Key words: histochemistry, horse, myopathy, polysaccharide complex, Zenker necrosis.

INTRODUCTION

Energetic metabolism alterations and muscular exercises induce in horse acute myopathy. The disease that have some non elucidated etiological factors is clinically and paraclinically represented by variable intensities locomotion inabiliy, myoglobinuria, hyperlactacidemy, uremia, and metabolic acidosis (Ghergariu et al., 1997). The acute myopathy was firstly reported 100 years ago and had different names influenced by some special circumstances and some dominant clinical signs. Also, there were utilized several names for this disease, such as: Setfast, Azoturia, Tying-Up, Paralytic myoglobinuria, Monday morning disease, and Paralytic myoglobinuria myopathy, and so on. In the last period, all presented entities represent in fact the same affection that actually is named equine rhabdomyolysis syndrome (ERS) with several clinical forms (Harris and Snow, 1986; Harris, 1998). From all E.R.S. forms it seems that acute rhabdomyolysis (AR) and recurrent exertional rhabdomyolysis (RER) have the most appropriate clinical and pathological signs for ERS (Art et al., 2000; Snow and Valberg, 1994).

AR take place during intense exercises (horse races and others exhaustive exercises). Due to these conditions, the horses are physically over-solicited getting out their effort capacity. In this particular situation into muscles take place an energetic crisis translated by acute ATP deficit together with lactate and others metabolites accumulation (ammoniac, ADP, inorganic phosphate).

The goal of the study was to establish some correlations between muscular lesions severity and glycogen stocking respectively utilizing capacity in two horses with AR.
MATERIAL AND METHODS

Biological material was represented by two geldings, which had about 6 respectively 8 years old. Both horses were private property resulted by crossing stray breed mares with heavy breed male horses. There was a sudden onset of AR symptoms, being the consequence of moderate training in both cases. Clinical signs were represented of movement disturbances, stiff gait, hind limbs muscle contracture, intense myoglobinuria, sterno-abdominal horse position followed by lateral one. Muscular enzymes (CK, ASAT, LDH) had increased values. The realized treatment was represented of intravenous fluid therapy with Lactate Ringer about 100 ml/kg/24h, flunixine meglumine 0,25 mg/kg x3/24h, acepromazin 0,04 mg/kg, triphosphadenum (Fosfobion®) 500 mg/horse/day and fortissimo B1 vitamin 1000 mg/horse/day. In both cases disease evolution was refractor to applied therapy and both horses died in two respectively four days from disease onset.

Necropsy revealed passive lungs congestion, hypostatic lungs edema, myoglobinuria, nephrosis, and large areas with acute muscular dystrophy localized into pelvic, spinal, and lumbar muscles. To realize histochemical investigations were harvested muscular samples of 5 mm thickness from mean gluteus, semitendinosus and semimembranosus muscles. There were selected these muscles because of their macroscopically feature observed into necropsy. On the other hand, specialty literature indicate the same muscle groups for acute rhabdomyolysis which is closely related with some particular changes of muscular glycogen properties (McGowan et al., 2003; Valberg et al., 1992; Valberg, 1995; Valentine, 1996; Valentine et al., 1997).

For histopathology exam harvested muscular samples were fixed in 10% formalin. The samples were processed using paraffin technique and longitudinally sectioned to about 6 µm and stained by Goldner trichrome method.

The muscular pieces designated for histochemistry exam were fixed in Carnoy mixture during 3 hours and embedded into paraffin and sectioned longitudinally to about 6 µm. To realize sections contrast was utilized PAS reaction. To control non modified muscular glycogen has been done dimedon blocking reaction and α-amylase digestion. Using microscopy were noticed muscular glycogen charging and its comportment to α-amylase action.

RESULTS AND DISCUSSIONS

Histopathology exam revealed the myofiber structure homogeneity and massive interfibrillar edema. These changes indicate initial vascular permeability disturbances and illustrate the AR onset. Due to vascular permeability disturbances interfibrillar edema is accentuated, which could be also notified in interfibrillar space too. Myofibrillar lesions are completed by myofibrillolysis followed by granule-vacuolar dystrophy and granule-hyaline complexes formation.

Histochemistry revealed existence of PAS positive material inside all myofibers cytoplasm. There are quite large quantitative variations of this material between different myofibers, aspect that was also indicated by PAS reaction intensity. In very affected myofibers are present some PAS positive blocks of varied size together with some granule-hyaline degeneration areas (Fig. 1, 2). All these muscular cells have some clear disruptions in some regions (Fig. 3). Control reactions with α-amylase made obvious both the presence of normal glycogen (that could be metabolized) and intense PAS positive polysaccharide material resistant to α-amylase lytic activity (Fig. 1-3). The non-metabolizable polysaccharides develop slowly (in many years) by irreversible combination of muscular glycogen (structurally normal) with sarcoplasm proteins (Valentine et al., 1997). In this manner obtained
glycogen-protein complexes are resistant to the action of α-amylase or to some other glycolysis enzymes. Physiological consequence of this particular situation is decreased bioavailability of modified glycogen that is necessary for both aerobe and anaerobe metabolic pathways involved in ATP synthesis (Erin et al., 2005). In this situation there is a large discordance between increased energetic myofibers requirement due to physical exercise and ATP availability delivered by two metabolic pathways. Limited muscular reserves of creatine phosphate together with glycolysis enzymatic system inefficacy generate acute energetic crisis in affected muscle. Severe energetic deficiency due to non-metabolizable glycogen-protein complexes deposited into sarcoplasm could be considered the main factor involved at least in acute myodystrophy lesions onset.

In studied cases was noticed a particular disposing of metabolizable glycogen and amylase-resistant polysaccharide complexes. Comparing the results obtained by simple PAS reaction with samples resulted by PAS reaction combined with α-amylase, in histological slides was observed a significant reduction of metabolizable glycogen in intense affected myofibers. In myofibers with large granule-hyaline degeneration and necrotic processes were met large amounts of amylase-resistant PAS positive material, which is in fact non-metabolizable glycogen. On the other hand, in myofibers that had large quantities of glycogen sensitive to the action of α-amylase lesions weren’t noticed or were discrete. Thus, it is quite clear that rhabdomyolysis lesions severity in studied cases were directly influenced of large amounts of amylase-resistant polysaccharide. Intramuscular presence of amylase-resistant polysaccharide complexes is actually considered to be the pathognomonic feature for polysaccharide storage myopathy (PSSM) in horses (Delfine and Benamon-Smith, 2002; Quinoz-Rothe et al., 2002; Valentine et al., 1998; Valentine, 1999; Valentine et al., 2000).

Fig. 1. Abnormal glycogen represented by amylase-resistant polysaccharide complexes into myofibers – PAS reaction combined with α-amylase digestion, 400x.

Fig. 2. Amylase-resistant polysaccharide complexes distribution into myofibers, and inter-myofibers edema – PAS reaction combined with α-amylase digestion, 400x.

The massive presence of PAS positive amylases-resistant material in both cases indicates that acute rhabdomyolysis clinical signs and consecutive muscular lesions are the consequence of PSSM. The first report of this kind of myopathy had been made in Quarter breed horses (Valberg et al., 1992;
Valberg, 1995), being later described in other horse breeds too (Delfine and Benamom-Smith, 2002; Valentine, et al., 1997; Valentine, 1999; Valentine et al., 2000;).

On national plan, PSSM was observed in horses used for work and resulted by crossing heavy breed horses with stray breed mares (Mircean, 2004). An important aspect is that some authors (Valentine, et al., 1997) highlight the necessity of long period of time to be formed polysaccharide complexes (at least 10 years) into myofibers. In our cases were identified amylase-resistant polysaccharide complexes in horses younger than 10 years, such as 6 respectively 8 years. This situation represented by polysaccharides complexes forming precocity in examined horses could be explained by an increased myofibers susceptibility to the action of low insulin quantities.

![Fig. 3. Degenerative and necrotic lesions located into myofibers that contains amylase-resistant polysaccharides complexes, intermyofibrillar edema – PAS reaction combined with α-amylase digestion, 400x.](image)

This particular situation could be proved by intravenous glucose and insuline tolerance tests (De La Corte et al., 1999; Mircean et al., 2005a,b). In this manner results massive normal glycogen stock into myofibers which is going to combine with sarcoplasm proteins generating glycogen-protein complexes. These complexes are resistant to the action of glycolysis enzymes resulting energetic crisis and acute rhabdomyolysis. Initially was suspected to be the consequence of some genetic disturbances of major enzymes involved in glycogen metabolism (glycogen sintethasys, 1,4-1,6 transglucosilasis, glycogen phosphorilasis). There aren’t enough dates about this. In the last period had been identified a mutant gene GYS1 placed into 10th chromosome. McCue et al. (2009) mention GYS1 gene involvement in persistent glycogen synthesis leading to abnormal polysaccharide complexes formation. GYS1 gene had been identified in 588 pure breed horses (ex. Percheron, Belgian, Appaloosa, Quarter
Horse) and in common breed horses, but wasn’t identified in Arabian horse breed (Valberg et al., 2009).

In both examined mixed breed horses the certain cause of acute rhabdomyolysis syndrome was PSSM, the results being highlighted by increased quantities of amylases resistant PAS positive polysaccharide complexes observed by histochemical exam. Due to recent results regarding genetic origin of PSSM are necessary further investigations to realize genetic prophylaxis in acute rhabdomyolysis.

CONCLUSIONS

Muscular lesions encountered in two horses affected of acute rhabdomyolysis were represented by massive interfibrillar edema, myofibrillolysis followed by granule-vacuolar dystrophy and granule-hyaline complexes formation.

Using histochemical technique, in affected myofibers from mean gluteus, semitendinosus and semimembranosus muscles were notified \( \alpha \)-amylase sensitive glycogen and amylase resistant polysaccharide complexes.

Myofibers lesions gravity was strictly related to no metabolizable glycogen quantities. The most severe lesions were noticed in myofibers with large quantities of amylase-resistant polysaccharide complexes disposed like PAS positive blocks. Muscular fibers charged with normal \( \alpha \)-amylase sensitive glycogen hadn’t had rhabdomyolysis.

In examined horses the muscular presence of the amylase-resistant polysaccharide complexes is a pathognomonic feature for PSSM which represents the primary cause for acute rhabdomyolysis syndrome in investigated cases.

REFERENCES


